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NEW PHYSIOLOGIC RACES OF FLAX RUST [1]

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INTRODUCTION

The results obtained from investigations of physiologic specialization in flax rust (*Melampsora lini* (Pers.) Lév.) during the years 1935 to 1938, inclusive, have rendered inadequate the key for the identification of races presented by the writer in an earlier paper (*1*)[3] and have vitiated some of the conclusions concerning the prospects for developing rust-immune varieties of flax through hybridization. The varieties of common flax (*Linum usitatissimum* L.) formerly reported as immune from all races of flax rust have been found to be susceptible to one or more of the races collected since 1935. Consequently, the number of flax varieties giving a differential reaction to the physiologic races of flax rust (*M. lini*) has been greatly enlarged. The results of investigations made since 1935 are reported in this paper.

MATERIAL AND METHODS

Methods of obtaining and propagating collections of flax rust, of inoculating and incubating flax plants, and of classifying host reactions and types of infection were described by the writer in 1935 (*1*). All results reported were obtained on flax plants approximately 30 days old, grown in the greenhouse at Fargo, N. Dak., during the winter months November to March, inclusive. The greenhouse was kept at a temperature of about 14° C. at night and 18° to 20° during the day. A light day of 16 hours was maintained during the period of pustule formation by supplementing daylight with artificial illumination from 200-watt Mazda bulbs. Final readings of the classes of host reaction and types of rust infection were made 10 to 15 days after inoculation, depending upon the effect of temperature and light on the rapidity of pustule development.

EXPERIMENTAL RESULTS

NEW DIFFERENTIAL VARIETIES OF FLAX

In order to differentiate the races of *Melampsora lini* collected since 1935, it has been necessary to add three varieties, Argentine (C. I. 462),[4] Bombay (C. I. 42), and Ottawa 770 B (C. I. 355), to the former list of differential varieties. These three varieties have been either immune from or susceptible to every race of rust thus far isolated, under all conditions encountered in the greenhouse.

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[2] The writer is indebted to A. C. Dillman, associate agronomist, Division of Cereal Crops and Diseases, for supplying seed of 150 or more varieties, for the preliminary grouping of varieties as shown in the tables, and for a critical reading of the manuscript. The writer is also indebted to A. C. Arny, associate agronomist, Minnesota Agricultural Experiment Station, and to José Vallega, of the Instituto Fitotécnico, Llavallol, Argentina, for supplying seed of a number of varieties.

[3] Italic numbers in parentheses refer to Literature Cited, p. 591.

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases.

The original strain of Williston Brown (C. I. 803) was lost. This strain was susceptible to wilt (*Fusarium lini* Bolley) and produced little seed even under favorable climatic conditions, so that no reserve of seed was accumulated. However, several single-plant selections from this strain were grown in the greenhouse during the winter of 1935–36 and from these a strain was secured that has considerable wilt resistance in addition to a more resistant reaction to the races of rust that were differentiated by the original strain of Williston Brown. This new strain (C. I. 803–1) was substituted for the former one. It has the necrotic (type 2) reaction to races to which it is resistant, and is not completely susceptible to many of the races collected in North America. The (type 3) uredia developed normally until they began to produce spores. After 2 or 3 days, development stopped, the pustules ceased to sporulate, and the leaves dried up. To other races it was highly susceptible; the uredia produced spores profusely for a period of 10 days to 2 weeks.

VARIABILITY IN REACTION OF DIFFERENTIAL VARIETIES OF FLAX

It has been customary in classifying rust reaction of crop plants growing under field conditions to take into consideration both the type and the number of pustules. Myers (*6*) and Vallega (*9*) followed this method in their classifications of the rust reactions of flax varieties. However, Hart (*2*) has shown that environmental conditions such as the temperature during the period the plants are in the moist chamber, the temperature during the remainder of the incubation period, and any condition that affects the vigor of the host plant, such as light intensity, temperature, moisture, and mineral nutrition, may alter not only the type but also the number of pustules. These conclusions of Hart have been verified repeatedly in studies made by the writer. In addition, it was observed that apparently identical changes in environmental conditions produced variable responses in different varieties and that the amount and viability of the inoculum affected the reaction of some varieties. The impracticability of accurately controlling the amount of inoculum, and the difficulties in spreading the spores evenly over all the inoculated areas and of rigidly controlling all phases of the environment to which the plant is subjected following inoculation render an attempt to classify degrees of resistance and susceptibility ineffectual. Attempts to differentiate too finely between degrees of resistance and susceptibility may lead to confusion and to a misunderstanding of results obtained at different localities or under variable conditions. A record of observations made on the reactions of the differential varieties when grown under a range of seasonal conditions in the greenhouse at Fargo may assist in the interpretation of results obtained in other localities.

The data presented in this paper were obtained during the winter months under the conditions previously described. However, the reaction range of the differential varieties to races of *Melampsora lini* collected locally has been studied throughout the year. On the basis of these studies with the 24 races of *M. lini* thus far differentiated, the differential varieties have been divided into 4 groups according to the stability of their general reaction under varying conditions and the range of their reaction to given races. These groups are as follows: (1) Stable reaction, always either immune or susceptible; (2) slightly variable reaction, immune, resistant, or susceptible; (3) slightly vari-

able reaction, a wide and almost continuous reaction range with many intermediates; and (4) variable and often intermediate reaction, a wide reaction range with certain races.

The differential varieties classed as having stable reaction have been either immune from or susceptible to every race of rust thus far isolated under all conditions and consequently have made ideal differentials. These varieties are J. W. S.[5] (C. I. 708–1) and three varieties, namely, Argentine (C. I. 462), Bombay (C. I. 42), and Ottawa 770 B (C. I. 355), that have been added to the list of varietal testers in order to differentiate the new physiologic races of rust.

The three varieties placed in the group having a slightly variable reaction have been immune, resistant, or susceptible to each of the 24 races of flax rust and have been satisfactory as differentials under all seasonal conditions. These varieties, Abyssinian (C. I. 701), Akmolinsk (C. I. 515–1), and Williston Golden (C. I. 25–1), have the chlorotic type of resistance (type 1) in which the uredia are minute to small and scattered in chlorotic areas of the leaves with but little necrosis of adjacent tissue. The distinction between susceptible and resistant-to-immune reaction in Abyssinian and Akmolinsk has been so wide under all conditions that there has been no difficulty in classifying their reaction. Under very favorable growing conditions for flax, the resistant-minus reaction (type 1 to 1+) of Williston Golden approached a susceptible-minus reaction (type 1+ to 3) but did not approximate it closely enough to be confusing.

Buda (C. I. 270–1), the only differential variety placed in the third group, had the most diverse reactions of all varieties tested. Its reaction range extended from immunity, through most of the intermediate stages, to a high degree of susceptibility. However, its range of reaction to any single race of rust was relatively narrow. Under adverse conditions for growth of flax the infection type on Buda sometimes approached that next lower in the scale of susceptibility. When conditions were such that the plants were growing thriftily, no difficulty due to seasonal conditions was encountered in identifying races differentiated by Buda.

The differential varieties in the fourth group had, to many races of rust, an intermediate reaction that was sensitive to changes in environmental conditions, so that considerable care was necessary in differentiating between degrees of resistance or susceptibility. To the races that they differentiated, these varieties had a relatively stable reaction. Two of these varieties, Kenya (C. I. 709–1) and Williston Brown (C. I. 803–1), had the necrotic type of resistance (type 2), in which the inoculated areas of the leaves became necrotic before the uredia were fully developed and the incompletely developed pustules were usually aggregated near the margins of necrotic areas. Isolated pustules were usually well developed but became prematurely surrounded by a necrotic zone. The reaction of "pale blue crimped"[6] (C. I. 647) to races to which it had an intermediate infection type was peculiar in that this variety became more resistant when growing vigorously under favorable conditions and more susceptible when weakened or retarded by unfavorable growing conditions.

The reaction of Argentine (C. I. 705) to races 1 to 14, inclusive, has been tabulated (*1*); but, since this variety had the variable reaction

[5] J. W. S. are the initials of J. W. Stewart, the originator of the variety.
[6] The word "crimped" is here used to describe the petal margins, which are incurved and somewhat wavy.

of group 4 and since it has not been necessary to use it in the key for the identification of physiologic races, it has been dropped from the list of differential varieties.

NEW PHYSIOLOGIC RACES OF FLAX RUST

Ten physiologic races of flax rust, in addition to the 14 previously described by the writer (*1*), have been differentiated by the type of infection produced on 11 varieties of cultivated flax.[7] All varieties that have been tested, including many that formerly were considered immune from flax rust (*1, 3, 4, 6*), have been found susceptible to 1 or more of the races isolated since 1935. A revised key to the races of *Melampsora lini* follows.

KEY

	Race
Buda resistant:	
Williston Golden resistant:	
Akmolinsk resistant:	
Williston Brown resistant	10
Williston Brown susceptible	1
Akmolinsk susceptible:	
J. W. S. resistant	5
J. W. S. susceptible	7
Williston Golden susceptible:	
Akmolinsk resistant:	
"Pale blue crimped" resistant	11
"Pale blue crimped" susceptible	6
Akmolinsk susceptible	20
Buda semiresistant:	
Williston Golden resistant:	
Akmolinsk resistant:	
J. W. S. resistant	17
J. W. S. susceptible	15
Akmolinsk susceptible:	
Abyssinian resistant	3
Abyssinian susceptible	18
Williston Golden susceptible:	
Akmolinsk resistant	14
Akmolinsk susceptible	23
Buda susceptible:	
Williston Golden resistant:	
Akmolinsk resistant:	
J. W. S. resistant:	Race
Kenya resistant	4
Kenya semiresistant	12
J. W. S. susceptible	13
Akmolinsk susceptible:	
J. W. S. resistant	8
J. W. S. susceptible	16
Williston Golden susceptible:	
Akmolinsk resistant:	
J. W. S. resistant:	
Bombay resistant	2
Bombay susceptible	24
J. W. S. susceptible	9
Akmolinsk susceptible:	
J. W. S. resistant:	
Ottawa 770 B resistant	19
Ottawa 770 B susceptible	22
J. W. S. susceptible	21

The characteristic reaction of seedling plants of 11 differential flax varieties inoculated with 24 physiologic races of *Melampsora lini* and grown in the greenhouse during the winter months at Fargo, N. Dak., is given in table 1.

It will be noted in table 1 that several of the races of flax rust isolated since 1935 have a wider varietal range than any of the 14 races previously described (*1*). The races of rust having greater virulence, as measured in terms of the number of differential varieties on which they produced a susceptible reaction, were obtained from Minnesota, North Dakota, Oregon, and South America.

Three of the six newly differentiated races of flax rust, collected in Minnesota or North Dakota, were more virulent than any of the races previously collected in this area. Race 16 was the first one isolated to which Buda, Akmolinsk, and J. W. S. were all susceptible and the first race collected in this area to which Abyssinian was not resistant.

[7] Since the preparation of this manuscript, two reports of work done by Straib in Germany have been published (*7, 8*). Straib differentiates eight physiologic races apparently different from those previously described by the writer. While several of his new races resemble somewhat certain races described in the present paper, the exact interrelations of these cannot be determined at present since he varied the technique employed by the writer and used additional host testers.

TABLE 1.—*Reaction of 11 differential varieties of Linum usitatissimum to 24 physiologic races of Melampsora lini*

Variety	C. I. No.	Reaction [1] to physiologic race No.—																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Buda	270–1	R+	S−	SR	S	R+	R+	I	S	S−	R+	R+	S−	S−	SR	SR	S	SR	SR	S	I	S	S	SR	S−
Williston Golden	25–1	R	S	R+	R+	R−	S	R+	R+	S−	R	S	R	R−	S	R	R+	R+	R	S	S	S	S	S	S
Williston Brown	803–1	S−	S−	S−	S−	S−	S−	R	S−	S−	R	S−	S−	S−	S−	S−	S−	S−	S	S	S	S	S	S	S−	S−
Akmolinsk	515–1	R+	R+	S	R+	S	R+	S	S	R+	R	R+	R+	R+	R+	R+	S	S−	S	S	S	S	S	S−	R+
J. W. S.	708–1	I	I	I	I	I	I	S	S	S	I	I	I	S	S	S	S	I	S	S	I	S	S	I	I
"Pale blue crimped"	647	S−	S−	S−	S−	SR	S−	S−	S−	S−	S−	S−	S−	S−	S−	S−	S−	S−	S−	S	S	S	S	S−	S−
Kenya	709–1	R+	R+	SR	R	R+	R	R+	SR	R	S−	I	SR	SR	R+	R	S−	R	S−	S	S	S	S	R	R
Abyssinian	701	I	I	R	I	R	I	R	S	I	I	I	I	I	I	I	R+	I	I	I	S	I	I	S−	I
Argentine	462	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	S−	I	I	I	S	I	I	I	I
Ottawa 770 B	355	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	R+	I	I	I	S	I	I	I	I
Bombay	42	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	S−	I	I	I	I	I	I	I	S

[1] Plus and minus signs indicate somewhat greater or less resistance or susceptibility than the letter designating the host reaction. The letters signify the following: I, Immune; R, resistant; SR, semiresistant; and S, susceptible.

Race 23 was the only one collected in the midwest seed-flax-producing area to which both Williston Golden and Akmolinsk were susceptible. Bombay, which had been immune from every rust collection previously tested, was susceptible to race 24, collected in North Dakota in 1938. Races 15, 17, and 18 had only minor differences in reaction from those previously described. Both Buda and Williston Golden were more resistant to race 15 than to race 9. The resistance of Akmolinsk differentiated race 17 from race 3. The moderate susceptibility of Abyssinian to race 18 differentiated this race from race 3.

The single uredial collection obtained from Oregon, designated race 21, produced a susceptible or semiresistant reaction on all the differential varieties previously used (*1*). The three added differentials, Argentine, Bombay, and Ottawa 770 B, were immune from race 21. This was the only North American race, with the exception of race 8, collected in Oregon in 1934, to which Abyssinian was highly susceptible.

Physiologic races 19, 20, and 22, obtained only from South America, possessed infection potentialities distinct from all North American races. Every variety used in differentiating physiologic races of flax rust was either highly susceptible to or immune from these South American races. The Argentine flaxes, formerly considered immune from rust (*1*, *2*, *3*, *4*, *6*), were highly susceptible to races 19, 20, and 22. Bombay, J. W. S., and Ottawa 770 B were immune from race 19; Bombay, Buda, J. W. S., and Ottawa 770 B were immune from race 20; and Bombay and J. W. S. were immune from race 22. The fact that race 22 was found able to attack Ottawa 770 B, which is immune from all other races, is of special importance, as previously this variety had been used as a rust-immune parent in breeding immune varieties of flax in the United States (*4*). Bombay and J. W. S. were found to be immune from all three races of rust from South America. However, Bombay was susceptible to race 24, from North Dakota, and J. W. S. was susceptible to several other North American races.

Buda was previously reported (*1*) resistant to race 7, but in subsequent tests has been consistently immune, and it is so classified in table 1.

GEOGRAPHIC DISTRIBUTION OF RACES OF FLAX RUST

Although no systematic survey has been made for the collection and identification of physiologic races of *Melampsora lini*, determinations have been made of collections from various sources. During the 4-year period from 1935 to 1938, 81 race determinations were made in North American urediospore collections. In addition, race determinations were made on two collections of viable telia obtained from South America. Germinating teliospores were used to inoculate flax plants and aecia were developed. A susceptible variety, Bison (C. I. 389), was inoculated with aeciospores from a single aecium, and race determinations were then made by using the urediospores thus produced to inoculate the differential varieties. From one of these telial collections two physiologic races were identified, and from the other collection four physiologic races were identified.

Physiologic races 1 to 18, inclusive, and 21, 23, and 24 were obtained from different localities in North America, and races 19, 20, and 22 were obtained from South America. The distribution of these races and the number of times each race was collected in each location are shown in table 2.

TABLE 2.—*Geographic distribution of physiologic races of Melampsora lini and number of times each race was identified in collections, 1935–38*

Year and source of collection	Isolates of physiologic race No.—																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
1935:																								
Canada [1]	1																							
Minnesota			3	3										1	1	1	3							
North Dakota			2	1					1			2					10							
Uruguay [2]	4																		1	1				
1936:																								
Minnesota																								
North Dakota	2		2	2													1							
Oregon [3]			1																		1			
1937:																								
Argentina [4]			1																1	1		1		
Minnesota	4		3		4	6						1		5			1	1					1	
North Dakota	2		1		1	1								3			1							
1938:																								
North Dakota	1	1																						1
Texas [5]														1										
Total	14	1	13	6	5	7	0	0	1	0	0	3	0	10	1	1	16	1	2	2	1	1	1	1

[1] Collected by W. G. McGregor at Ottawa, Ontario, Canada.
[2] Collected by J. G. Fischer and Albert Boerger, La Estanzuela, Colonia, Uruguay.
[3] Collected by B. B. Robinson at Astoria, Oreg.
[4] Collected by José Vallega, Llavallol, Argentina.
[5] Collected by E. S. McFadden, at Victoria, Tex.

There appeared to have been little change since the earlier report (*1*) in the races of rust that predominated in the midwest seed-flax-producing area. The ones with a more limited varietal range continued to be predominant, although there were races present that had a wider varietal host range. Since the principal commercial seed-flax variety, Bison, is susceptible to all known races of flax rust, there probably was no natural selective survival going on among the more widely virulent strains, such as might take place if a variety were grown that was resistant to the present prevailing races but susceptible to those races having a wider varietal range.

Races 19 and 20 were segregated from a telial collection obtained from Uruguay. These 2 races also predominated in the aecial isolates made from the telial collection received from Argentina, and races 3 and 22 also were obtained from these latter isolates. Races 19, 20, and 22 were obtained only from South America and therefore special care was taken to prevent their escape from the greenhouse. Tests were conducted only during the winter months and all plant parts, pots, and soil were steamed before being discarded.

In 1938, a uredial collection sent from a Texas flax field sown with Bison seed obtained from North Dakota was identified as race 14. This race was prevalent in Minnesota and North Dakota flax fields the preceding year and the Texas infection probably originated from telia sown with the seed.

Race 24 was isolated from a row of heavily rusted Bombay sown in the field at Fargo, N. Dak., in 1938. This seed was obtained from José Vallega, who reported this variety immune in Argentina (*9*).

REACTION OF VARIETIES OF FLAX

One of the objects of a study of physiologic specialization in parasitic organisms is to facilitate the production of immune or resistant varieties of crop plants. A knowledge of the regional and world-wide reaction of the parental material to the disease-producing organism is essential, especially when the highly specialized rusts are involved. In the data presented in an earlier paper (*1*), based on a study of North American collections of *Melampsora lini*, so few of the flax varieties showed differential response to the physiologic races then isolated that it was considered more desirable, in recording the reaction of varieties, to list them according to type of flax rather than according to their response to the different races of flax rust. The varietal specificity of several of the more recently isolated races of flax rust has made it desirable to group the flax varieties on the basis of their differential reaction to the races of rust thus far isolated. On this basis the 201 varieties and strains of flax that have been tested with the 16 principal physiologic races, Nos. 1, 2, 3, 4, 5, 7, 8, 9, 10, 16, 19, 20, 21, 22, 23, and 24, have been grouped as follows: (1) Varieties and strains susceptible to all these races; (2) those having the specific immunity of (a) Argentine, (b) Ottawa 770 B, (c) J. W. S., and (d) Bombay; (3) those having varying degrees of resistance and susceptibility; and (4) those too heterogeneous to classify as to predominant type of reaction. The several races that were not included have pathogenic properties differing but slightly from one or more of those used in these tests and the additional information would not have warranted the increased cost in time and effort their use would have entailed. Cases where the variety was not pure for rust reaction and instances

where a variety consistently exhibited an intermediate type of reaction have been noted.

The varieties and strains of flax predominantly susceptible to all 16 physiologic races of *Melampsora lini* that were used are listed in the following tabulation. Of the 201 varieties and strains of flax tested, 91 were in this group.

Varieties of flax susceptible to physiologic races 1, 2, 3, 4, 5, 7, 8, 9, 10, 16, 19, 20, 21, 22, 23, and 24 of Melampsora lini and immune from none

Group and variety	C. I. No.
Dehiscent flax (*Linum usitatissimum crepitans*):	
From Siberia	295
From Germany	469
From Ukraine	506
Do	507
Seed flax (*Linum usitatissimum*):	
Petals broad, flat:	
Abyssinian:	
Abyssinian Yellow-seed	36
Abyssinian	300
Do	[1] 302
Abyssinian from Egypt	[1] 380
Abyssinian from Kenya, East Africa	707
American and European:	
Blue-flowered:	
Bison	389
Bolley No. 32–1823	[2] 754
Buda	326
Linota	244
Minnesota 25–410	421
Minnesota 25–202	447
Minnesota 25–221	423
Minnesota 25–245	446
Minnesota 25–107	438
Minnesota 25–241	458
North Dakota Resistant 5	411
North Dakota Resistant 52	275
North Dakota Resistant 114	489
North Dakota Resistant 714	399
North Dakota Resistant 726	412
North Dakota 40016	428
Pale blue	387–1
Redwing	320
Slope	274
Winona	481
Argentine×Saginaw	660
White-flowered:	
Blanc	323–3
Diadem	[3] 321
Ottawa white-flowered	24
Tammes white	329
Tammes white, type 10	774
Tammes yellow seed	331
Tammes yellow seed, type 13	777
Seed flax—Continued.	
Petals broad, flat—Continued.	
American and European—Con.	
Pink-flowered:	
Deep pink	648
Long No. 4	400
Long No. 66	337
Do	719
Long No. 83	354
Pale pink	[2] 173–1
Do	[2] 173–3
Pale pink (M25–228)	479
Tall pink	451–3
Tammes pink	334
Tammes pink, type 8	772
Tammes deep pink	336
Tammes deep pink, type 9	[1] 773
Argentine:	
Capa	[3] 720
De Sanare	[3] 1002
Klein 10 e	[3] 887
Rosario	[3] 316
Indian:	
Howard and Khan (*5*):	
var. *luteum*, type 1	
var. *cyaneum*, type 8	
var. *purpureum*, type 11	
var. *album*, type 15	[4]
var. *agreste*, type 22	
var. *meridionale*, type 25	
var. *pratense*, type 28	
Russian:	
Billings	[3] 184
Crimean (No. 2237) [5]	[3] 563
Fergana (No. 401) [5]	512
Novelty	[3] 140
Samarkand (No. 2238) [5]	[3] 514
Hybrid:	
Petals narrow, margins incurved or crimped:	
Crimped white (Minn. 25–125)	392
Crimped white (Minn. 29–55)	[6] 685
Ottawa 829–C	391
Pale blue	[3] 646
Tammes crimped white	330
Tammes crimped white, type 11	775

See footnotes at end of table.

Varieties of flax susceptible to physiologic races 1, 2, 3, 4, 5, 7, 8, 9, 10, 16, 19, 20 21, 22, 23, and 24 of Melampsora lini and immune from none—Continued

Group and variety	C. I. No.
Seed flax—Continued.	
Petals broad, flat—Con.	
Hybrid—Continued.	
Petals narrow, etc.—Con.	
Tammes type 12 (seeds brown)	776
Yellow seed B	325
Fiber flax (*Linum usitatissimum*):	
Blue-flowered:	
Althausen	628
Do	[1] 630
Dalgonetz	498
F. I.[7] No. 3	[3] 694
Hercules	
J. W. S.	[1] 388
Fiber flax—Continued.	
Blue-flowered—Con.	
Liral Crown	882
Peerless	695
Saginaw	449
Stormont Cirrus	881
Stormont Gossamer	[3] 883
White-flowered:	
Blenda	
Concurrent	801
Friesland white	56–1
Minnesota 25–64	420
Pinnacle	693
Saginaw white	448

[1] Some plants resistant to or immune from each race.
[2] Susceptible (8−) to most North American races. 8− denotes a susceptible-minus host reaction in which the uredia are large but less abundant than in a susceptible reaction and in which there is considerable distortion and chlorosis or necrosis of the infected leaf tissues.
[3] Some plants resistant to or immune from North American races.
[4] Susceptible (8−) to race 10.
[5] Obtained from Dr. N. I. Vavilov, Union of Soviet Socialist Republics, in March 1930.
[6] Some plants immune from all races except race 22.
[7] F. I. = Fiber investigations.

While there was no absolute correlation between flax type and rust reaction, there appeared to be definite relationships. The four strains of dehiscent flax (*Linum usitatissimum* var. *crepitans* Bönningh.) were uniformly susceptible. Most American and European seed flax varieties, as well as most fiber flax varieties, were susceptible to all races of rust. However, there were a few varieties in these groups that possessed resistance to or immunity from certain races. Several varieties of Abyssinian and of Indian type were highly susceptible, as also were a number of varieties having narrow incurved or crimped petals. Most of the Argentine and Russian flaxes listed as predominantly susceptible were not pure for rust reaction and contained varying percentages of plants immune from North American races but susceptible to races 19, 20, and 22 from South America.

It is possible that some of the varieties listed as susceptible to all races may give a differential reaction when tested with additional races of rust. No European rust collection has been tested, but the report by Henry (*3*) that flax varieties resistant or immune in The Netherlands were susceptible in Minnesota would indicate the existence of varieties susceptible to North American races but resistant to or immune from some European races. The variety Rosario (C. I. 316) and two selections of "pale pink" (C. I. 173–1 and 173–3) that formerly were reported (*1*) as resistant to North American races have been found to be somewhat susceptible and have been included among the susceptible varieties listed in the tabulation on pages 583–584.

It has been pointed out that four of the differential varieties, Argentine, Bombay, J. W. S., and Ottawa 770 B, have been either immune from or susceptible to each of the 24 races of flax rust. The reaction of these varieties suggests that each possesses a factor or factors for immunity from certain races of rust not common to the other three varieties. The studies that have been made on the inheritance of immunity from rust in flax bear this out. Henry (*4*) found that immunity from races of North American rust was dominant

and conditioned by a single factor in the varieties Bombay and Ottawa 770 B and by two factors in a selection of Argentine type. Myers (*6*) verified Henry's results in regard to Ottawa 770 B. He also found that the immunity of an Argentine flax (C. I. 712) could be explained by assuming the existence of two dominant factors governing rust reaction, one factor determining immunity and the other near-immunity from a North American rust collection. Unpublished data obtained by the writer have shown that immunity in J. W. S. is conditioned by a single dominant factor. Flax varieties having rust reactions similar to that of each of the differential varieties Argentine, Ottawa 770 B, J. W. S., and Bombay have been grouped in the following tabulations to facilitate inventories of potential parental material for breeding rust-immune flax.

Flaxes of Argentine type were immune from all North American rust collections but highly susceptible to races 19, 20, and 22 from South America. Varieties having either or both of the factors for immunity or near-immunity from North American rusts as found by Henry (*4*) and Myers (*6*) are in this group. Most of these varieties are definitely of the Argentine type or hybrids in which an Argentine type of flax was a parent. However, three pink-flowered American seed flaxes, one Indian, three Mediterranean, and two Russian type flaxes had the rust reaction characteristic of Argentine type varieties. Varieties and strains having the reaction of the Argentine type flaxes are listed in the following tabulation.

Varieties of seed flax (Linum usitatissimum) susceptible to physiologic races 19, 20, and 22 of Melampsora lini, and immune from races 1, 2, 3, 4, 5, 7, 8, 9, 10, 16, 21, 23, and 24

Group and variety	C. I. No.
American and European:	
Pink-flowered:	
Bolley Golden	[1] 644
Bolley No. 32–1822	750
Smoky Golden	751
Argentine:	
Argentine (N. Dak. 1742)	[1] 342
Argentine (Minn. 25–343)	[2] 417
Argentine (Minn. 25–341)	462
Argentine (Minn. 25–362)	472
Argentine (Minn. 25–330)	[1] 690
Argentine (Minn. 25–361–1)	[1] 691
Argentine (Selection C. I. 379–3)	[2] 692
Argentine (Minn. 25–323)	[2] 705
Biglow	[2] 414
Capa 10	[3] 721
Capa 11	722
Kenya	[4] 706
Do	[2] 709
Light Mauve	[5] 379–1
Lineta Z 195	[6] 1003
Lino Grande	[1] 381–2
Long No. 5	[2] 466
Malabrigo	[1] 346
Do	696
Rio	280

Group and variety	C. I. No.
Indian:	
Howard and Khan (*5*):	
var. *pulchrum*, type 34	——
Mediterranean:	
Beladi	[6] 377
Giza	[1] 378
Morocco	[2] 376–2
Russian:	
Damont	3
Newland	[2] 188
Hybrid:	
Argentine × Saginaw	651
Do	652
Do	653
Do	[7] 654
Do	656
Do	657
Do	[7] 658
Do	[7] 661
Do	664
Do	667
Bolley 37–5310	877
Bolley 37–5242	878
Natural hybrid, Rosario	820
Selection Bison × (160 × 179)	818
Selection (19 × 112) × 19	819

See footnotes at end of table.

Varieties of seed flax (Linum usitatissimum) susceptible to physiologic races 19, 20, and 22 of Melampsora lini, and immune from races 1, 2, 3, 4, 5, 7, 8, 9, 10, 16, 21, 23, and 24—Continued

Group and variety	C. I. No.
Hybrid—Continued.	
Selection of C. I. 385 (19 × 112)	823
Selection of C. I. 496 (160 × 179)	824
Selection of C. I. 385 (19 × 112)	825
Walsh	[1] 645

[1] Some plants resistant to North American races.
[2] Some plants resistant or susceptible to North American races.
[3] Resistant to races 16, 21, and 23.
[4] Some plants resistant to all races except 22.
[5] Resistant to North American races.
[6] Some plants immune from races 19 and 20.
[7] Some plants susceptible to North American races.

The high proportion of varieties having the Argentine type of rust reaction, but not pure for immunity from North American races of flax rust, may be due (1) to a relatively high percentage of natural crossing in these varieties or (2) to an immune and to a near-immune or resistant factor for rust reaction, as pointed out by Myers (*6*).

The varieties with the reaction of Ottawa 770 B, i. e., immune from all races except race 22 from South America, are listed in the following tabulation.

Varieties of seed flax (Linum usitatissimum) susceptible to physiologic race 22 of Melampsora lini, and immune from races 1, 2, 3, 4, 5, 7, 8, 9, 10, 16, 17, 18, 19, 20, 21, 23, and 24

Group and variety	C. I. No.
Petals broad, flat:	
Argentine:	
Bolley 37–5012	[1] 874
Klein 11–o	1004
Lineta Z 176	885
Pergamino selection	884
Tammes pale blue	[1] 333
Petals narrow, margins incurved or crimped:	
Ottawa 770 B	355
Minnesota hybrids:	
Saginaw × Ottawa 770 B	675
Do	676
Do	677
Do	679
Petals narrow, etc.—Continued.	
Minnesota hybrids—Continued.	
Saginaw × Ottawa 770 B—Continued.	
Do	682
Do	684
Do	685–1
Do	686
Do	687
Do	[2] 681
Winona × Ottawa 770 B	672
Do	673
Do	674
Do	716

[1] Some plants susceptible to all races.
[2] Some plants susceptible to races 19 and 20.

Most of these varieties were hybrids in which Ottawa 770 B was a parent. The origin of Tammes pale blue and of the four varieties from Argentina having rust reaction similar to that of Ottawa 770 B was not available.

The four varieties that were immune from the same physiologic races as the differential selection of J. W. S. are listed in the following tabulation.

Varieties of flax (Linum usitatissimum) susceptible to physiologic races 7, 9, 16, and 21 of Melampsora lini, and immune from races 1, 2, 3, 4, 5, 8, 10, 19, 20, 22, 23, and 24

Group and variety	C. I. No.
Seed flax:	
Petals broad, flat:	
Italia Roma	[1] 1005
Tammes light blue	332
Tammes light blue, type 2	766
Fiber flax:	
Blue-flowered:	
J. W. S.	708–1

[1] Not highly susceptible to races 7, 9, 16, and 21.

Tammes light blue types were identical and pure for rust reaction. Italia Roma (C. I. 1005), in addition to being immune from the races to which the J. W. S. selection and Tammes light blue types were immune, was somewhat resistant to the races to which these varieties were susceptible. The reaction of Italia Roma suggested that it had a modifying factor for resistance in addition to the factor for immunity possessed by the differential strain of J. W. S.

The eight varieties listed in the following tabulation had previously been considered immune from all races of flax rust but were susceptible to race 24 found in field plots at Fargo, N. Dak., in 1938. These are all Indian types or hybrids in which an Indian type flax was a parent. The 12 varieties listed in the following tabulation and in that immediately preceding were the only varieties tested that were not predominantly susceptible to race 22 from South America.

Varieties of seed flax (Linum usitatissimum) susceptible to race 24 of Melampsora lini, and immune from races 1, 2, 3, 4, 5, 7, 8, 9, 10, 16, 19, 20, 21, 22, and 23.

Group and variety	C. I. No.
Indian:	
Howard and Khan (*5*):	
var. *minor*, type 29	
var. *commune*, type 46	
var. *commune*, type 48	
var. *commune*, type 53	
Bombay	42
Punjab	20
Hybrid:	
Saginaw × Bombay	671
Vallega 2181–1	

The varieties that had reactions intermediate between "immune" and "susceptible" to one or more races of *Melampsora lini* are listed in table 3. The seven differential varieties that were neither immune from nor susceptible to each race are included in this table. Two selections of Buda × (19×112) (C. I. 821 and 826) had reactions identical with those of the differential line of Buda. The reaction of Abyssinian (C. I. 511) was similar to that of the differential selection of Akmolinsk (C. I. 515–1). The other varieties listed in table 3 deviated from each of the differential varieties in their reaction to one or more physiologic races of rust. This suggests that these varieties possess factors or combinations of factors modifying rust reaction not possessed by any of the 11 differential varieties listed in table 1. It is probable that several of these varieties may be of value in differentiating new physiologic races of flax rust.

The reaction to North American races of rust of several varieties listed in table 3 was found to be affected by small variations in environment and thus was extremely variable. The reactions of "pale pink" (C. I. 649), Indian type 55, "pale blue" (C. I. 176), and "pale blue crimped" (C. I. 647) were especially subject to modification by a variable environment.

TABLE 3.—*Reaction of varieties of seed flax (Linum usitatissimum) having intermediate differential reactions to physiologic races of Melampsora lini*

Group and variety	C. I. No.	Predominant reaction [1] of 30-day-old plants to physiologic race No.—															
		1	2	3	4	5	7	8	9	10	16	19	20	21	22	23	24
Petals broad, flat:																	
Abyssinian:																	
From Fergana	511	R	R	S	R	S	S	S	R	R	S	S	S	S	S	S	R
Abyssinian	701	I	I	R	I	R	R	S	I	I	S−	S	S	S	S	S−	I
American and European:																	
Blue-flowered:																	
Buda	270–1	R	S−	SR	S	R+	I	S	S−	R+	S	S	I	S	S	SR	S−
Williston Brown	803–1	S−	S−	S−	S−	S−	R	S−	S−	R	S−	S	S	S	S	S−	S−
Pink-flowered:																	
Lethbridge Golden	23	S	S	S	S	S	R	S	S	R	S	S	S	S	S	S	S
Pale pink	649	R	R	R	R	R	R	R	R	I	R	S−	S−	R	S−	SR	R
Williston Golden	25–1	R	S	R+	R+	R−	R+	R+	S−	R+	R+	S	S	S	S	S	S
Argentine:																	
Argentine (Minn. 25–323)	705–1	SR	SR	SR	SR	SR	S−	SR	SR	SR	SR	S	S	SR	S	SR	SR
Bolley (37–5090)	879	R	R	R	R	R	S−	R	R	R	R	S	S	R	S	R	R
Kenya	709–1	R+	R+	SR	R	R+	R+	SR	R	R+	R+	S	S	SR	S	R	R
Indian:																	
Howard and Khan (*5*):																	
var. *commune*, type 55		R	R	R	R	R	S−	S	S−	R	S	S	S	S	S	S−	S
var. *campestre*, type 68		I	I	I	I	I	I	I	I	I	R	S−	S−	R	S−	I	S
var. *sativum*, type 121		R	R	R	R	R	S−	R	R	I	R	S−	S−	S	S−	R−	S
Russian:																	
Akmolinsk	515–1	R+	R+	S	R+	S	S	S	R+	R+	S	S	S	S	S	S	R+
Pale blue	176	R	R	R	R	R	R	S−	S−	R	S	S	S	S	S	R	S−
Hybrid:																	
Selection Buda×(19×112)	821	R	S−	SR	S	R+	I	S	S−	R+	S	S	I	S	S	SR	S−
Do	826	R	S−	SR	S	R+	I	S	S−	R+	S	S	I	S	S	SR	S−
Selection (19×112)×Buda	822	I	I	I	I	I	I	I	I	I	I	S	S	I	S	I	S−
Selection Buda×(19×112)	827	I	I	I	I	I	I	I	I	I	I	S	S	I	S	I	S−
Petals narrow, margins incurved or crimped:																	
Pale blue crimped [2]	647	S−	S−	S−	S−	SR	S−	S−	S−	S−	S−	S	S	S−	S	S−	S−

[1] I=immune, R=resistant, SR=semiresistant, and S=susceptible. Plus and minus signs indicate somewhat more or less resistance or susceptibility than the letter designating the host reaction.

[2] Immune from physiologic race 11.

The varieties in which the rust reaction was so mixed that it was difficult to determine the predominant reaction with each race are listed in the following tabulation. Six of the 11 varieties listed in this table were pure for susceptibility to South American races 19, 20, and 22.

Varieties of seed flax (Linum usitatissimum) too heterogeneous to classify as to predominant type of reaction to physiologic races 1, 2, 3, 4, 5, 7, 8, 9, 10, 16, 19, 20, 21, 22, 23, and 24 of Melampsora lini in greenhouse tests

Group and variety	C. I. No.
Mediterranean:	
Crete	[1] 31–1
Cyprus	689
Russian:	
Akmolinsk	[2] 520
North Caucasian	[3] 620
Winter:	
Roman Winter	[1] 470
Hybrid:	
Bolley (37–5066)	[1] 876
Long No. 125	[1] 356
Reserve × Morye (19×112)	486
Tammes, type 1 (common blue)	765
Tammes, type 3	767

[1] Susceptible to races 19, 20, and 22.
[2] Susceptible to races 19, 20, 21, and 22.
[3] Some plants immune from each race.

DISCUSSION

The production of rust-immune varieties of flax has been rendered more difficult by the discovery of new physiologic races of *Melampsora lini* to which all the varieties that had previously been considered immune were susceptible. Henry (*3*) noted that certain strains of Argentine flax, strains of Williston Golden, several varieties obtained from India, and Ottawa 770 B were immune in tests made in Minnesota. In his hybridization studies, he used selections of Argentine, Ottawa 770 B, and an Indian variety, Bombay, as supposedly rust-immune parents. In tests reported upon in this paper, all strains of Williston Golden were found to be highly susceptible to several races of flax rust prevalent in North America, while Bombay and several other Indian flaxes that were previously considered to be immune were susceptible to race 24 collected at Fargo, N. Dak. Ottawa 770 B and certain Argentine strains were immune from all races collected in North America but were susceptible to race 22 from South America. All of Henry's supposedly rust-immune hybrid strains that have been tested by the writer have been found to be susceptible to certain races and to have the reaction of the supposedly rust-immune parent. Myers (*6*) used Newland (C. I. 188) and a strain of Bolley Golden (C. I. 644), in addition to Ottawa 770 B and an Argentine selection, as immune parents in his studies on inheritance of rust reaction in flax. In the present studies all of these varieties were found to be susceptible to race 22 and also, with the exception of Ottawa 770 B, to races 19 and 20 from South America. Not one of the 201 varieties tested was immune from or resistant to all of the 24 races of flax rust thus far differentiated. These varieties had been selected for testing because of their diverse morphologic type, their agronomic importance, or their reported resistance to or immunity from rust. While all possibilities have not been exhausted there is a basis for doubt as to the existence of a variety of common flax immune from all races of rust.

There is a possibility, however, that a variety immune from all races of flax rust could be developed by hybridization. The varieties that apparently have the same factor for immunity as J. W. S. and

those that apparently have the same factor as Bombay were immune from races to which the Argentine type flaxes and Ottawa 770 B were susceptible. The latter varieties were, in turn, immune from those races of rust to which J. W. S. or Bombay were susceptible. No data exist as to combining in one variety these factors for immunity. Myers (*6*) found in a cross between two immune varieties, Newland and Ottawa 770 B, that immunity was conditioned by a single dominant factor in each variety and that the factors in the two varieties were not allelic. In the cross between the immune varieties Ottawa 770 B and Argentine selection (C. I. 438), his results suggested that C. I. 438 carried the same factor that conditioned immunity to Ottawa 770 B and also a factor for resistance that was allelic to the factor for immunity carried by Newland. The susceptibility of C. I. 438 to races 19 and 20, and the immunity of Ottawa 770 B from these races would indicate that Myers' suggestion of identical factors for immunity in these two varieties was not correct. The factor for immunity from the North American rusts used by Myers in C. I. 438 was probably allelic to the factor for immunity from North American rusts and races 19 and 20 from South America in Ottawa 770 B. This difference between the immune factors in these two varieties would not be apparent with the rusts used by Myers. If the factors for immunity carried by either Bombay or J. W. S. are not allelic to the factor for immunity carried by either Ottawa 770 B or Newland, it should be possible to develop a variety immune from all the known races of *Melampsora lini*.

There is a relatively wide range of flax varieties and types available for parental material having the resistance of the Argentine differential. Varieties in this group included American pink-flowered seed flaxes and Argentine, Indian, Mediterranean, Russian, and hybrid types. Choice of varieties available for parental material having the factors for immunity of the other three immune groups is relatively limited. Of the 20 varieties and selections having the immune reaction of Ottawa 770 B, 13 were Ottawa 770 B hybrids. Only 4 varieties had the immune reaction of J. W. S. Two of these were apparently identical strains of Tammes light-blue type 2. All 8 varieties that had the immune reaction of Bombay were Indian type flaxes or hybrids in which an Indian type flax was a parent.

Even if it is found possible to develop a strain of flax immune from all known races of *Melampsora lini*, the possibility of the rust hybridizing and developing a new race capable of attacking the immune flax should not be overlooked. In temperate regions, flax rust overwinters in the telial stage and the initiation of infection in the spring is dependent upon a natural hybridization process. It appears desirable to determine the inheritance of the factors governing different degrees of pathogenicity in the rust organism as well as those governing immunity in the host. Until this is done it seems desirable to exercise all possible precautions against the establishment in North America of the races attacking Ottawa 770 B, Newland, and Argentine flaxes. Similar precaution should be taken against the establishment in South America of races attacking Bombay and J. W. S.

SUMMARY

Ten new physiologic races of flax rust (*Melampsora lini*), in addition to the 14 previously reported, have been identified by the reaction of

11 varieties of flax. To differentiate the new races it was necessary to add 3 varieties, previously considered immune from rust, to the list of host testers. These are Argentine (C. I. 462), Bombay (C. I. 42), and Ottawa 770 B (C. I. 355).

All of the 201 varieties and strains of flax tested were susceptible to 1 or more of the 24 physiologic races of *Melampsora lini* that have been identified. These varieties and strains were selected for testing because of their diverse morphologic type, their commercial possibilities, or their reported resistance to or immunity from flax rust. Flaxes of Argentine type and Ottawa 770 B remained immune from all races of rust collected in North America but were susceptible to one or more of the races from South America.

Bombay and J. W. S. were immune from the races of rust obtained from South America but were susceptible to one or more of the North American races.

Pathogenicity tests indicate that each of the differential varieties Argentine, Bombay, J. W. S., and Ottawa 770 B possesses distinct factors governing immunity from specific races of flax rust. Of 201 varieties tested, 48 had the reaction of Argentine, 8 the reaction of Bombay, 4 the reaction of J. W. S., and 20 the reaction of Ottawa 770 B to 16 physiologic races of *Melampsora lini*.

The races of flax rust with a rather limited varietal range continued to predominate in the seed-flax-producing area of the Midwest despite the presence of a number of the races possessing a wider varietal range.

LITERATURE CITED

(1) Flor, H. H.
1935. physiologic specialization of melampsora lini on linum usitatissimum. Jour. Agr. Res. 51: 819–837, illus.

(2) Hart, Helen.
1926. factors affecting the development of flax rust, melampsora lini (pers.) lév. Phytopathology 16: 185–205, illus.

(3) Henry, A. W.
1926. flax rust and its control. Minn. Agr. Expt. Sta. Tech. Bul. 36, 20 pp., illus.

(4) ———
1930. inheritance of immunity from flax rust. Phytopathology 20: 707–721, illus.

(5) Howard, Gabrielle L. C., and Khan, Abdur Rahman.
1924. studies in indian oil seeds no. 2. linseed. India Dept. Agr. Mem., Bot. Ser. 12: 135–183, illus.

(6) Myers, W. M.
1937. the nature and interaction of genes conditioning reaction to rust in flax. Jour. Agr. Res. 55: 631–666, illus.

(7) Straib, W.
1939. zum epidemischen auftreten des leinrostes in ostpreussen. Nachrichtenbl. f. den Deut. Pflanzenschutzdienst 19: [49]–51.

(8) ———
1939. untersuchungen über den wirtsbereich und die aggressivität physiologischer rassen von melampsora lini (pers.) lév. Züchter 11: 130–136, 162–168, illus.

(9) Vallega, José.
1938. observaciones sobre la resistencia a la roya de algunos linos ensayados en el instituto fitotécnico de llavallol. Santa Catalina Inst. Fitotéc. Pub. No. 1, 34 pp. [From Rev. Argentina de Agron., t. 5, No. 1.]

239804—40——2

THE EFFECT OF SEVERAL CHEMICALS ON THE SWELLING AND THE CRUSHING STRENGTH OF WOOD[1]

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INTRODUCTION

It is common knowledge that wood shrinks or swells with a loss or gain in moisture below the fiber-saturation point. It is probably not so well known, or perhaps merely not realized, that changes in the strength of wood also result from various moisture equilibria. The effect of aqueous solutions of electrolytes and nonelectrolytes upon the swelling of wood and some other cellulosic materials has received an increasing amount of study, but very little research has been done to determine their effect on strength and the correlation of strength and swelling caused by aqueous solutions and organic liquids. Such study is undoubtedly of fundamental and practical importance and deserves further investigation.

The relation between moisture content and the mechanical strength of wood is well described by Wilson (*20*).[2] Most of the important literature on the swelling of cellulosic materials, including wood, has been adequately reviewed by Stamm (*17*). The swelling of cellulose and adsorption of aqueous solutions and some organic liquids are discussed. Gerritsen (*3*) reported that filter paper disintegrated in salt solutions, in the order of the lyotropic series, which were not sufficiently concentrated to disperse the cellulose; this was ascribed to swelling and low mechanical strength. Stamm found that swelling of wood was not influenced by the hydrogen-ion concentration except at values above pH 8 to 9 (*15*). Solutes in concentrated aqueous solutions displaced some of the water below the fiber-saturation point in wood (*15*, *17*, *18*). Katz (*6*) found that some nondissociated organic compounds also cause swelling of cellulosic materials beyond the water-swollen dimensions. Hasselblatt (*5*) determined the amount of swelling of oven-dry wood by several organic liquids such as ether, alcohol, creosote, glycerol, etc. The swelling of wood and of lignin by several other organic compounds has been reported by Saechtling and Zocher (*11*). Lignin was apparently more organophilic than cellulose. Sheppard and Newsome (*13*), in studying the adsorption of alcohols by cellulose, found an inverse relation between swelling and the carbon number of the normal alcohols. The same type of curve for adsorption of alcohols by wood was obtained by Stamm (*16*). Lüdke (*8*) found that the strength of regenerated cellulose fibers varied in different organic media.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 602.

MATERIALS

The chemicals that were used in this study were of two types: (1) Water-soluble inorganic and organic solids; and (2) organic liquids, principally the lower aliphatic alcohols. All the inorganic salts were of c. p. quality or better and were as follows: LiCl, $CaCl_2$, $MgCl_2$, $MnCl_2$, KCL, NaCl, KSCN, KI, KBr, and K_2SO_4. The carbon compounds used in aqueous solution were as follows: Urea, c. p.; chloral hydrate, U. S. P.; resorcinol, c. p.; pyrogallol, a. r.[3]; and tannic acid, U. S. P. (Mallinckrodt).

The organic liquids were: Methyl alcohol, a. r.; ethyl alochol, absolute; the alcohols *n*-propyl, isopropyl, *n*-butyl, isobutyl, and *n*-amyl (Eastman Kodak Co.); acetone, c. p.; ethylene glycol practical (Eastman); and benzene, c. p.

The wood used in this study was red pine (*Pinus resinosa* Aiton). Only the sapwood was used.

The blocks were measured with a jaw-type micrometer caliper calibrated to one one-thousandth of an inch.

PROCEDURE

Most of the aqueous solutions of inorganic salts were made to concentrations approaching their solubilities at 20° C. as given by the International Critical Tables (*10*). The Li, Ca, Mg, and Mn chlorides, however, are a little below their solubilities at 20°. Inasmuch as the solubility values of the critical tables could not be checked on several of the organic compounds, it was decided to round off the molal concentrations near their limits of solubility. In calculating the concentrations of these solutions the water held by the wet or green wood (necessary to obtain the dimensions on a green basis) has been included.

Ten blocks, each about 1 by 1 by 2 inches when green, were used in the test with a given liquid. Eight billets were sawed from one-half of the log and from each of these 3 to 6 series of 10 blocks were sawed with a circular miter saw. Defective blocks were discarded; the others were sandpapered smooth.

All of the blocks were dried slowly to a uniform moisture content of 2 percent during a storage period. The blocks which were to be placed in aqueous solutions, and the control blocks, were then permitted to gain moisture until they were in approximate equilibrium with the atmosphere, following which they were brought above the fiber-saturation point by immersion in water. After 4 days they were measured in the tangential, radial, and longitudinal directions. Throughout the experiments, measurements were taken at the same places each time. The blocks to be impregnated with aqueous solutions were brought back near the fiber-saturation point by vacuum and careful drying.

The blocks were impregnated as well as possible with the solution by forcibly submerging them and applying an intermittent high vacuum for several periods of approximately one-half hour each. Complete impregnation was not always obtained, especially with the highly concentrated solutions, but this was probably not a serious matter. Evaporation losses were restored in order to keep the solu-

[3] Analytical reagent.

tion at the proper concentration. The blocks and liquid finally were transferred to an airtight glass jar and kept at 25° C.

The blocks which were to be used with organic liquids and the oven-dry control set were dried at 106° to 107° C. for an average time of 12 hours. These conditions with blocks that were already very dry probably did not diminish appreciably the strength or hygroscopicity (*4*, *19*) of the wood. The seven blocks tested at 11.3 percent moisture content had been soaked in water, carefully oven-dried to verify the swelling values, and then rehumidified. Although the high vapor pressure of some of the organic liquids prevented the obtainment of a high vaccuum, saturation with the organic liquids was quite complete.

Five of the ten blocks in all sets were measured at weekly intervals after the first 3 to 4 weeks to determine when the swelling was complete. When no further change occurred they were tested for maximum crushing strength.

The compression tests were of the uniform rate of strain type which was approximately 0.035 inch per minute. The 10 blocks used in each test were so selected that as many positions as possible in the half-log were represented. Consideration was given to the radial distance into the log and the position of the vertical series of blocks in a tangential direction. The specific gravity of the water-soaked control blocks based on green volume and oven-dry weight was determined by the water-displacement method. The specific gravity varied from 0.38 to 0.41; the average was 0.39.

EXPERIMENTAL RESULTS

EFFECT OF ORGANIC LIQUIDS

The results of the tests for maximum crushing strength parallel to the grain are expressed in pounds per square inch of transverse area of the block at the oven-dry condition. This method gives the actual loss in strength suffered by a piece of wood of unit area under standard

TABLE 1.—*The effect of certain organic liquids and of water upon the swelling and maximum crushing strength of wood*

Liquid	Period of immersion	Maximum crushing strength per square inch based on oven-dry area			Swelling on oven-dry basis		
		Minimum value	Maximum value	Average value	Radial	Tangential	Cross-sectional
	Days	*Pounds*	*Pounds*	*Pounds*	*Percent*	*Percent*	*Percent*
Oven-dry wood		10,315	11,900	11,225			
n-Amyl alcohol	40	10,585	11,460	10,960	0.12	0.24	0.30
Isobutyl alcohol	41	8,990	10,730	10,220	.26	.47	.67
n-Butyl alcohol [1]	75	8,730	9,570	9,205	1.50	2.87	4.51
n-Butyl alcohol [1]	504	6,905	7,585	7,350	2.26	5.18	7.28
Acetone	41	7,555	8,660	8,130	2.77	6.14	8.90
n-Propyl alcohol	41	7,515	8,495	7,960	2.84	6.22	9.13
Isopropyl alcohol	40	4,750	5,560	5,260	3.44	7.04	10.71
Ethyl alcohol	41	6,170	7,150	6,615	3.52	7.78	11.53
Methyl alcohol	44	4,085	4,516	4,310	4.13	9.10	13.61
Ethylene glycol	44	4,175	4,540	4,330	4.06	10.77	15.29
11.3 percent water content [2]		6,430	7,320	6,715	1.73	3.20	5.04
Water-saturated		2,040	2,430	2,650	4.49	10.24	15.20

[1] 5 blocks used in this test.

[2] 7 blocks used.

conditions when it is treated with the various liquids. The ordinary method is to express the values based on the area at the time of the test. The amount of swelling expressed in percent of the oven-dry basis is given for the cross-sectional or transverse area of the blocks and for the radial and tangential directions. Longitudianal swelling was usually small; therefore, the cross-sectional swelling was nearly equal to the volumetric swelling (less than 0.2 percent difference in the extreme cases).

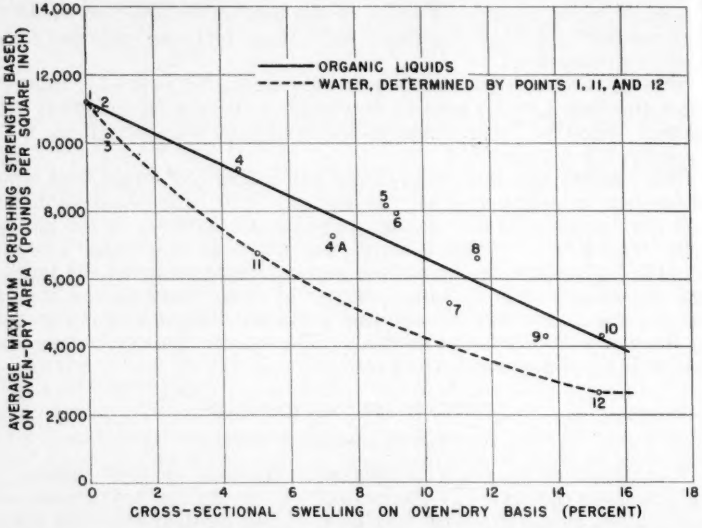

FIGURE 1.—The effect of various liquids upon the relationship between the crushing strength and swelling of wood: 1, Oven-dry wood; 2, *n*-amyl alcohol; 3, isobutyl alcohol; 4, *n*-butyl alcohol; 4A, *n*-butyl alcohol; 5 acetone; 6, *n*–propyl alcohol; 7, isopropyl alcohol; 8, ethyl alcohol; 9, methyl alcohol; 10, ethylene glycol; 11, 11.3-percent water content; 12, water-saturated.

It is evident from table 1 and figure 1 that the character of the liquid affects the swelling and the crushing strength of wood. The relative position of the compounds indicates that the degree of polarity was intimately associated with the swelling and strength of the wood. The polar alcoholic group of *n*–amyl alcohol, for example, was much less effective than the same group on a short-chain alcohol molecule. The higher alcohols which approach a hydrocarbon structure caused less swelling and less loss of strength. The effect of a pure hydrocarbon, benzene, on the swelling and strength of wood was determined by using a different sample of wood of the same species (results not given in table 1 or figure 1). The benzene was dried with sodium and distilled twice. Blocks impregnated with this liquid and kept immersed for 20 days showed no swelling and no statistically significant difference in strength from the oven-dry control blocks. This is what would be expected from consideration of the above generalization.

There is definite indication that the relationship between swelling and crushing strength for organic liquids is different from that for water (fig. 1). When one observes the difference between strength values in the two curves for any given amount of swelling, it is apparent that the organic liquids employed have, as a class, some distinctive action which does not weaken wood to the extent that water does for the same degree of swelling. Although the curve for water (broken line) was determined by only three points, it is believed to be sufficiently accurate for comparison inasmuch as the two extreme values were from matched control blocks and from the same 10 series of blocks that were used for the organic liquids. The curve is similar in general shape to the common moisture content-strength curve (*20*). Since swelling is a straight-line function of moisture content (*16*) they may be interchanged on the same axis.

The degree of significance of maximum crushing strength between certain sets of blocks whose normal deviates were in the lower range is shown in the following tabulation:

Test combinations:	Normal derivates
Methyl alcohol—ethylene glycol	0. 40
Acetone—*n*-propyl alcohol	1. 16
Oven dry—*n*-amyl alcohol	2. 16
Oven dry—benzene	. 33
KCL—K_2SO_4	3. 19
KBr—H_2O	. 89
$MgCl_2$—chloral hydrate	. 42
$CaCl_2$—chloral hydrate	1. 63
$MgCl_2$—$CaCl_2$	1. 16
NaCl—$MnCl_2$	1. 30
Li Cl—K_2SO_4	3. 10
Ki—KSCN	3. 20
$MgCl_2$—tannic acid	3. 58
Chloral hydrate—tannic acid	3. 06

Several values for the organic compounds are not statistically significant on the basis of maximum crushing strength. Those test combinations listed in the foregoing tabulation which show a normal deviate (t) of less than 2 fall in this class. The correlation index of crushing strength to swelling for the organic liquids was found to be 0.96 and the percent of the total variance of strength from the mean which was associated with swelling was almost 93 percent.

Isobutanol acted more like a hydrocarbon than the *n*-alcohol, whereas isopropyl acted in the opposite manner. The iso-alcohols also caused greater reduction in strength in proportion to the swelling effected, as shown by their position below the straight line, than the normal alcohols.

Two sets of values which represent different periods of immersion are given for *n*-butyl alcohol. All the sets of blocks except those in *n*-butyl alcohol had reached equilibrium at some time before 24 days, when the second measurements were made. These latter blocks continued to swell, and at the end of 75 days five of them were tested for strength; the other five were allowed to remain in the liquid. Figure 2 shows a linear relationship between time and swelling up to about 110 days after which the curve rapidly flattens out. The remaining five blocks immersed in the *n*-butyl alcohol had nearly the same cross-sectional swelling after 504 days as at 156 days, the percentages being 7.28 and 7.4 respectively. Constant temperature was not maintained after 100 days but apparently this had little effect.

In figure 1 the first point for butyl alcohol falls almost on the line, but the second is approximately 500 pounds below the expected value. The fact that the final swelling value is closer to the propyl alcohol than would be expected in view of the relative position to the amyl and isobutyl values, also suggests a specific action on the wood which is different from that of other alcohols.

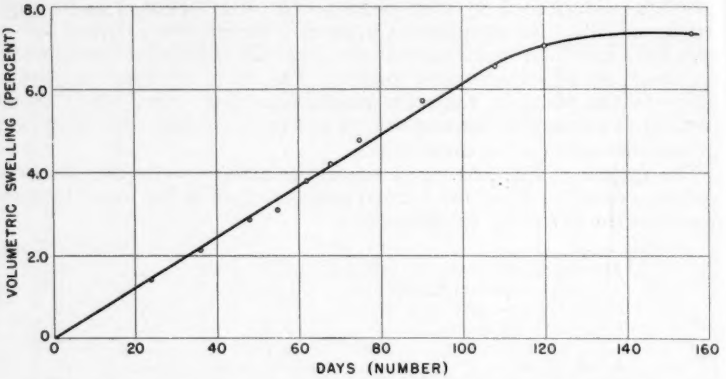

FIGURE 2.—Relation between the extent of swelling of wood and the time in which it was kept immersed in *n*-butyl alcohol.

TABLE 2.—*Effect of concentrated aqueous solutions of certain mineral salts and organic compounds as compared with water on the swelling and maximum crushing strength of wood*

Solute	Quantity per kilogram of water	Quantity per 100 gm. of water	Period of immersion	Swelling beyond water-swollen dimensions			Maximum crushing strength, per square inch, based on water-swollen dimensions			Change in average maximum crushing strength compared with water
				Radial	Tangential	Volumetric	Minimum	Maximum	Average	
	Equivalents	*Grams*	*Days*	*Percent*	*Percent*	*Percent*	*Pounds*	*Pounds*	*Pounds*	*Percent*
Water							2,040	2,430	2,300	
LiCl	18.00	76.3	31	+0.1	1.4	1.4	2,460	2,850	2,680	+16.5
$CaCl_2$	11.78	65.5	46	−.1	.9	.8	3,200	3,505	3,355	+46.0
$MgCl_2$	11.14	48.3	45	−.1	.6	.5	3,125	3,545	3,295	+43.3
$MnCl_2$	10.00	62.8	44	.0	.5	.4	2,775	3,010	2,880	+25.2
NaCl	6.1	35.7	50	.0	.0	.0	2,650	2,930	2,830	+23.0
KCl	4.6	34.3	49	.0	.1	.1	2,400	2,580	2,485	+8.1
KCNS	22.2	215.7	40	−.1	1.5	1.6	1,940	2,220	2,065	−10.2
KI	8.7	144.5	39	−.2	1.1	.9	1,885	2,105	1,975	−14.1
KBr	5.5	65.5	49	.1	.5	.6	2,175	2,455	2,340	+1.3
KCl	4.6	34.3	50	.0	.1	.1	2,400	2,580	2,485	+8.1
K_2SO_4	1.3	11.5	50	−.1	.1	.0	2,475	2,610	2,540	+10.4
Chloral hydrate	[1] 15.0	248.0	39	.2	2.2	2.5	3,060	3,505	3,270	+42.2
Tannic acid		59.0	36	.1	.4	.4	2,945	3,265	3,110	+35.2
Urea	[1] 15	90.0	45	−.2	1.5	1.3	1,510	1,710	1,605	−30.2
Pyrogallol	[1] 5	63.0	34	−.3	2.9	2.6	1,045	1,125	1,075	−53.2
Resorcinol	[1] 10	110.0	43	−.3	3.1	2.8	1,000	1,075	1,030	−55.2

[1] Molal concentration.

EFFECT OF AQUEOUS SOLUTIONS

Table 2 reveals an absence of a general correlation between swelling and crushing strength. All solutions except NaCl and K_2SO_4 caused appreciable swelling beyond the water-swollen dimensions. This was accompanied by various degrees of weakening or strengthening, depending upon the swelling agent. Neither loss nor gain of strength can be closely associated with the higher values of swelling when considering solutions of either electrolytes or nonelectrolytes. Table 2 shows that the entire series of chloride salts caused an increase in the crushing strength, up to 46 percent in the case of $CaCl_2$, as compared with the water-soaked control blocks. In this group there is a tendency toward a correlation between swelling and increase in crushing strength as one goes up the series of salts, until LiCl is reached. Apparently the strengthening effect of this salt is counterbalanced by a weakening factor, which may be caused by the lithium ion. The thiocyanate and iodide salts, usually considered to be good peptizers, caused a decrease in strength although not in proportion to the degree of swelling. From the bromide to the sulfate salts, a small increase in strength occurred.

The nonelectrolyte solutions also exhibited variable effects in crushing strength. Chloral hydrate and pyrogallol in the concentrations used caused about the same amount of volumetric swelling, but the former increased the strength by 42.2 percent whereas the latter decreased it by 53.2 percent. Chloral hydrate, pyrogallol, and resorcinol effected considerably more cross-sectional swelling than any of the inorganic salts.

In general, cross-sectional swelling followed the order of the lyotropic series for both the cations and the anions, with the Li and SCN ions in the lead positions. This order coincides fairly well with that of solubility, fractional volume occupied by the salt, relative vapor pressure, surface tension, and the general belief of the order of hydration of ions.

Radial shrinkage was manifested, usually, only when swelling in the tangential direction was relatively large. It was greatest when the strength of the wood was below normal. The "plastic" condition of such wood may have contributed to this result by permitting compensation for excessive tangential swelling, for it was observed, while testing the blocks, that those exposed to resorcinol and pyrogallol solutions had no point of abrupt failure; the beam remained in balance with very little adjustment of weight for a far longer time than was true with blocks from the NaCl solution, for example. The failure with urea was more abrupt.

DISCUSSION

The proof of an inverse correlation between swelling and maximum crushing strength for organic liquids was in accordance with expectations. There was no indication as to why the wood was stronger in organic liquids than when swollen with water or water vapor to the same volumetric change. The cause may be associated with molecular constitution and size and the resultant differences in adsorption and orientation on the micellar surfaces, with the accompanying variation in shift of secondary valence forces from micelle to liquid. Unless

one knows something about the character of the compound, therefore, swelling is only a very rough guide for predicting strength values.

Stamm (*15*, *17*) suggests that the theory of electrical repulsion is applicable to explain swelling in organic liquids, and cites as evidence the same approximate order of swelling of wood in benzene derivatives and their order of dipole moments and zeta potential (*9*) at a cellulose interface. Although this may hold for those compounds, a similar relationship did not obtain with the alcohols. If the alcohols only are considered it is noted that there is a regular decline in negative zeta potential from methyl to isopropyl alcohol, after which the sign is positive and the magnitude is variable. There is no consistent relationship between the electric moments (*14*) of the organic liquids used and swelling or strength of the wood. The same is true with surface tension, mainly because the surface-tension values of the alcohols are nearly the same. A general relationship of viscosity of the liquid to swelling and strength of wood is indicated, especially with the alcohols. The higher alcohols have the higher viscosities. Acetone and benzene deviate greatly and several of the stronger swelling agents do not fall in the expected order. Therefore, viscosity does not seem to be a dependable criterion of the effect of any liquid on wood.

The dielectric constants of these liquids (from 15.8 for *n*-amyl alcohol to 41.2 for ethylene glycol at 20° C.) are practically in the same order as the degree of swelling. When swelling is plotted against dielectric constants a nearly parabolic curve is obtained if one disregards the position of isobutyl alcohol which would increase the curvature. It cannot be predicted, of course, that any type of compound with a given dielectric constant can be satisfactorily interpolated in such a graph. It may well be that each class will have a different numerical range of constants which will represent the two extremes of swelling power.

The data show that the lithium, thiocyanate, and iodide ions gave a decrease in strength values. This suggests a relationship to their well-known top position in the lyotropic series for peptizing power and might counteract to a varying extent the strengthening action of a decreased water equilibrium in the wood caused by high salt concentration. It is noted that chloral hydrate caused greater swelling than any of the salts and also markedly increased the crushing strength. Hence, mere excessive swelling of wood does not alone cause a decrease in strength.

Investigators [4] have reported that phenol caused a decrease in strength of wood; this was believed to be due to the swelling of lignin. Pyridine and phenol were more strongly adsorbed from solution by by lignin than by cellulose. This may explain why resorcinol and pyrogallol caused considerable swelling and loss of strength of wood, especially the observed property of flowage or apparent plasticity of the wood when subjected to its maximum compressive srtength. Adsorption by cellulose of phenols from dilute solutions increased with the number of hydroxyl groups on the molecule (*2*). This coincides in a qualitative way with the data in table 2 in which a 5-molal solution of pyrogallol caused nearly as much swelling and

[4] GRASSBY, H. G., JONES, G. D. O., POTTER, G. J. C., and YORSTON, F. H. THE SWELLING OF WOODS BY ORGANIC COMPOUNDS. Forestry Prod. Lab. Canada, Quarto. Rev. July 1933, pp. 22-38, illus. 1933. [Mimeographed.]

loss of strength as a 10-molal solution of resorcinol. Constituents or relatives of tannin were strongly adsorbed (*2*).

Koehler (*7*) observed that heating wet wood was likely to cause some radial contraction and tangential expansion. The results reported herein on radial shrinkage and nonswelling are somewhat in contradiction to those obtained in other work (*14*). The data show that radial shrinkage is correlated much more with those liquids that reduce strength than with those that tend to increase it. This suggests that the shape and structure, both fine and gross, of a piece of wood may be important factors in determining (1) what stresses are created by excess swelling and (2) to what extent it will yield or adjust when weakened or strengthened in the process. In considering also the results of other investigators (*1*, *7*, *12*), it seems evident that enough data are not available from which to draw a definite conclusion as to the cause of radial shrinkage or nonswelling, especially for excessive swelling of blocks as compared with sections or fragments of wood.

It is probable that the findings of this study are of importance in their bearing upon the structural uses of wood that has been subjected to fire-retarding treatment because of the relatively large amount of chemicals that must be injected and the variety of chemicals that can be used. The strength changes, if any, caused by inorganic preservative treatment probably are of low magnitude for the reason that only small quantities of chemicals are needed.

It is apparent that the results of this experiment add to the complexity of the relationship between swelling and strength of wood. Clearly, there is no constant and proportional relation, either direct or inverse, which is independent of the chemical agents. More research is necessary before an attempt can be made to answer the many questions which a study of the data brings to mind.

SUMMARY

A definite inverse relationship was found between the swelling of wood and its maximum crushing strength when the swelling agents were organic liquids, chiefly alcohols. For the same degree of swelling, the blocks in organic liquids were stronger than those swollen with water vapor or liquid. The lower alcohols caused the greater swelling and loss of crushing strength, the higher alcohols the least. Benzene did not cause swelling or affect appreciably the strength of oven-dry wood. More than 120 days were required for swelling of wood in *n*-butyl alcohol to reach equilibrium.

The amount of swelling caused by concentrated solutions of inorganic salts was in general agreement with the solubility of the salts and the lyotropic series of ions as found by other workers. Some of these solutions increased the strength of wood; a few decreased it. The chloride series caused an increase. In the organic group, urea, resorcinol, and pyrogallol markedly decreased the crushing strength of wood, whereas chloral hydrate, which swelled wood the most, and tannic acid decidedly increased the strength. Very little radial swelling occurred beyond the green dimension and in several aqueous solutions radial contraction took place. The degree of swelling does not appear to be a dependable criterion of the change in crushing strength of wood; specific effects of the chemicals often dominate the results.

LITERATURE CITED

(1) BEISER, W.
1933. MIKROPHOTOGRAPHISCHE QUELLUNGSUNTERSUCHUNGEN VON FICHTEN- UND BUCHENHOLZ AN MIKROTOMSCHNITTEN IM DURCHFALLENDEN LICHT UND AN HOLZKLÖTZCHEN IM AUFFALLENDEN LICHT. Kolloid Ztschr. 65: 203–211, illus.

(2) BRASS, KURT, and GRONYCH, OTTO.
1937. DIE AUFNAHME VON PHENOLEN UND PHENOLKARBONSAUREN DURCH ZELLULOSE (MODELLVERSUCHE ZUM STUDIUM DER AUFNAHME VON GALLOTANNINEN DURCH ZELLULOSE). Kolloid Ztschr. 78: 51–68, illus.

(3) GERRITSEN, D. J.
1936. UITEENVALLEN EN OPLOSSEN VON CELLULOSE IN GECONCENTREEDE ZOUTOPLOSSINGEN. Chem. Weekbl. 33: 405–406.

(4) GREENHILL, W. L.
1936. STRENGTH TESTS PERPENDICULAR TO THE GRAIN OF TIMBER AT VARIOUS TEMPERATURES AND MOISTURE CONTENTS. Austral. Council Sci. & Indus. Res. Jour. 9: 265–276, illus.

(5) HASSELBLATT, MEINHARD.
1926. DER WASSERDAMPFDRUCK UND DIE ELEKTRISCHE LEITFÄHIGKEIT DES HOLZES IN ABHÄNGIGKEIT VON SEINEM WASSERGEHALT. Ztschr. f. Anorgan. u. Allg. Chem. 154: [375]–385, illus.

(6) KATZ, J. R.
1933. THE LAWS OF SWELLING. Faraday Soc. Trans. 29: 279–300, illus. [Discussion, pp. 299–300.]

(7) KOEHLER, ARTHUR.
1933. EFFECT OF HEATING WET WOOD ON ITS SUBSEQUENT DIMENSIONS. Amer. Wood Preservers' Assoc. Proc. 29: 375–388, illus.

(8) LÜDKE, WERNER.
1929. DER EINFLUSS VON FLÜSSIGKEITEN AUF GEFORMTES ZELLULOSEHYDRAT. Kolloid Ztschr. 47: 341–351, illus.

(9) MARTIN, WILLIAM MCKINLEY, and GORTNER, ROSS AIKEN.
1930. STUDIES ON ELECTROKINETIC POTENTIALS. V. INTERFACIAL ENERGY AND THE MOLECULAR STRUCTURE OF ORGANIC COMPOUNDS. I. ELECTROKINETIC POTENTIALS AT CELLULOSE-ORGANIC LIQUID INTERFACES. Jour. Phys. Chem. 34: [1509]–1539, illus.

(10) NATIONAL RESEARCH COUNCIL.
1926–33. INTERNATIONAL CRITICAL TABLES OF NUMERICAL DATA, PHYSICS, CHEMISTRY AND TECHNOLOGY . . . 7 v. and index. New York.

(11) SAECHTLING, HJ., and ZOCHER, H.
1934. UNTERSUCHUNGEN ÜBER DIE FEINSTRUKTUR DES HOLZES. EINFUHRUNG UND TEIL I. DIE VOLUMENQUELLUNG VON FICHTENHOLZ UND VON LIGNIN IN VERSCHIEDENEN MEDIEN. Kolloid Beihefte 40: [413]–448, illus.

(12) SCHWALBE, CARL G., and BEISER, WILLI.
1933. DIE QUELLUNG VON HOLZ DURCH WASSER UND WÄSSRIGE LÖSUNGEN. Papier Fabrikant 31 (Ver. der Zellstoff u. Papier Chem. u. Ingenieure): 655–667, illus.

(13) SHEPPARD, S. E., and NEWSOME, P. T.
1932. THE SORPTION OF ALCOHOL VAPORS BY CELLULOSE AND CELLULOSE ACETATES. Jour. Phys. Chem. 36: [2306]–2318, illus.

(14) SMYTH, CHARLES PHELPS.
1931. DIELECTRIC CONSTANT AND MOLECULAR STRUCTURE. 214 pp., illus. New York.

(15) STAMM, ALFRED J.
1934. EFFECT OF INORGANIC SALTS UPON THE SWELLING AND SHRINKING OF WOOD. Amer. Chem. Soc. Jour. 56: 1195–1204, illus.

(16) ———
1935. SHRINKAGE AND SWELLING OF WOOD. Indus. and Engin. Chem. 27: 401–406, illus.

(17) ———
1936. COLLOID CHEMISTRY OF CELLULOSE MATERIALS. U. S. Dept. Agr. Misc. Pub. 240, 91 pp.

(18) STAMM, ALFRED J.
1937. TREATMENT WITH SUCROSE AND INVERT SUGAR. Indus. and Engin. Chem. 29: 833–835, illus.
(19) ——— and HANSEN, L. A.
1937. MINIMIZING WOOD SHRINKAGE AND SWELLING. Indus. and Engin. Chem. 29: 831–833, illus.
(20) WILSON, T. R. C.
1932. STRENGTH-MOISTURE RELATIONS FOR WOOD. U. S. Dept. Agr. Tech. Bul. 282, 88 pp., illus.

THE INFLUENCE OF TEMPERATURE, MOISTURE, AND FOOD UPON THE DEVELOPMENT AND SURVIVAL OF THE SAW-TOOTHED GRAIN BEETLE [1]

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INTRODUCTION

The saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (family Cucujidae), has been reported from many parts of the world as a pest of a wide variety of stored-food products. It is the grain insect most frequently sent in by Minnesota farmers for identification. Many observations in regard to its life history have been made and numerous short accounts of its distribution, habits, and biology are available in various reports. Prior to 1926 the brief biological notes of Chittenden (*6*)[2] were almost the only ones of a quantitative nature that had been published. In 1926 Back and Cotton (*3*) reported their observations made under ordinary room conditions with no attempt to control either temperature or moisture. The object of the present work was to obtain more definite information on the life history of this insect over a considerable range of controlled conditions in relation to temperature, food, and atmospheric moisture.

METHODS OF STUDYING DEVELOPMENT

Mass populations of saw-toothed grain beetle adults were reared on the moist, balanced food mixture recommended by Haydak (*8*) and held in a constant temperature chamber at 32° C. and 75 percent relative humidity. Under these conditions it was possible to obtain large numbers of the insect in a relatively short time. Several hundred beetles were collected and placed in an oviposition chamber, a pint fruit jar containing a small amount of the food mixture and several irregularly broken pieces of a large cork stopper. The cork had previously been softened and made pliable by moistening and autoclaving.

The eggs, which are normally laid in cracks and crevices of food material, are covered with an adhesive substance which causes them, when first deposited, to adhere to each other and to the food particles. Cork was found to provide a very suitable surface for the females to oviposit on and from which to retrieve the eggs. Following oviposition, the pieces of cork were removed and the adhering beetles shaken into the culture jar for future use. The eggs adhering to the cork were readily observed by means of a binocular microscope and were picked off with a fine moistened brush. They were then placed individually on the inner walls of 2-dram vials some distance above a small supply of food. Nearly half of the hatching experiments had

[1] Received for publication June 28, 1939. Paper No. 1723 of the Scientific Journal series, Minnesota Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 614.

to be discarded because of injury to the eggs during handling. The larvae left the empty eggshells attached to the walls, where they could be seen easily, making it unnecessary to locate and disturb the minute, white, fragile larvae among the food particles.

The adult beetles are very excitable, and as handling of them was unavoidable before each oviposition period considerable time elapsed before the deposition of eggs actually occurred. Oviposition periods of 2, 4, and 6 hours were tried, but it was found necessary to extend the period to 10 hours. The beetles were placed in the oviposition chamber at midnight to facilitate the measuring of the development periods. Including the time required to retrieve them, the eggs were from 1 to 14 hours old before they were exposed to the various experimental conditions. Very few eggs were found to have been laid in the first 4 hours, however, so it is assumed that the majority of them were not over 6 to 7 hours old.

Eggs were placed singly in individual 2-dram vials which contained sufficient food and were stoppered with perforated corks covered with small pieces of silk bolting cloth. The vials containing the food had previously been conditioned at the desired temperature and humidity for at least 36 hours. Groups of 30 vials, each vial containing one egg with food, were placed in small desiccators held at constant temperature. Various known concentrations of sulfuric acid in water were used to control the atmospheric moisture within the desiccators. The acid concentrations required to give the desired atmospheric conditions were calculated from a graph taken from Wilson (*16*). The desiccators were at no time removed from the cabinets during the experimental period, and the vials were out only for a few minutes daily during the critical periods, preliminary tests indicating approximately when observations were to be made. It is believed that the atmosphere of the vials reached an equilibrium with the atmosphere in the desiccators within a reasonably short time since the desiccators were small and the vials were but a short distance above the acid solutions.

The various stages of the insect were exposed to temperatures of 15°, 20°, 25°, 30°, 35°, and 40° C. in cabinets controlled by toluene-mercury thermostats which did not vary more than $\pm 0.5°$. At least three replications were made in each category.

Two levels of atmospheric moisture or saturation deficiency were at first chosen, 5 mm. and 12.5 mm. As the work progressed it was thought desirable to provide a wider range of moisture conditions and a third level, 22.5 mm. of saturation deficiency, was added.

Three rather different types of food commonly infested by the saw-toothed grain beetle were used, namely, rolled oats, English walnuts, and raisins. A sufficient quantity of each for the entire experiment was obtained at the beginning of the study. Each vial contained enough of the food material to more than support the insect, but not enough for the larva to burrow into and become covered. The larva, therefore, was more or less exposed to the atmosphere at all times. Raisins, because of their hygroscopic nature, did not lend themselves to some of the conditions of the experiment. The young larvae frequently became enmeshed in the sticky syrup produced by the raisins and in such cases there was a high mortality.

Two measures of moisture were utilized in this work, saturation deficit and relative humidity. The first is a measure of the evapora-

tive power of the atmosphere and, being uniform at different temperatures, may be applied to the study of the direct effect of atmospheric moisture upon insect growth. At a 5-mm. saturation deficiency, for example, the rate of evaporation is theoretically constant at the temperatures 35° 30°, 25°, and 20° C.

Atmospheric moisture, on the other hand, is absorbed by food materials in proportion to the relative humidity of the air. The relative humidities corresponding to a 5-mm. saturation deficiency at 35°, 30°, 25°, and 20° C. are 88, 84, 79, and 72 percent respectively. In preliminary tests rolled oats exposed in desiccators at these temperatures and humidities for 4 days and then dried at 105° for 24 hours showed a moisture content on a dry-weight basis of about 21.6, 22.8, 18.8, and 15.9 percent respectively. In the analyses of Atwater and Bryant (*1*) soft-shelled walnuts averaged 2.5 percent moisture. Finely divided soft-shelled walnuts exposed to relative humidities of 46.7 and 88.0 percent at 35° for 5 days and then dried over sulfuric acid for 10 days gave approximate moisture-content values of 1.7 and 6.6 percent. Walnuts appear to be less hygroscopic than rolled oats owing possibly, to their high oil content and to their reduced surface.

If moisture were held constant in terms of relative humidity, regardless of temperature, the food moisture content would be constant, but the rate of evaporation from the surface of the insect would then be variable because of the differences in saturation deficiency. By holding one value constant the other will be variable. It is realized, therefore, that in studying the effects of temperature alone, the moisture relationships are not entirely in agreement. Although the rates of evaporation are constant, the moisture content of the food material differs slightly at each temperature, and the changes in the rate of development cannot be said to be due entirely to the effects of temperature. The larvae in a condition of higher relative humidity, because of a higher percentage of water in the food, will be able to replace water loss with greater ease than those at a lower relative humidity. Much greater effects, in general, can be attributed to temperature, and it is believed that errors due to the variation in food moisture content are not very great. On walnuts, the moisture content of which is little affected by wide variations in humidity, the error is probably much reduced.

In these experiments the walnuts were divided to permit accessibility of the food to the newly hatched larvae. Schwardt (*14*) observed that finely divided foods of high oil content were detrimental to larval development. He stated that when the larvae crawl upon or through divided oily food they are brushed on all sides and become coated with a thin film of oil. If the food particles are larger the larvae can pass through the interstices and only touch the food with their tarsi and part of the ventral surface of the abdomen. In this study it was found that ground walnuts were fatal to the larvae whereas chopped walnuts had no detrimental effects.

In the statistical procedure the following formula of Fisher (*7*) was used:

$$S.\ E.=\frac{\sqrt{\frac{S(x^2)-S(x)\bar{x}}{N-1}}}{\sqrt{N}}.$$

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The formula for determining the error of the difference between two means is as follows:

$$\frac{(\bar{x}_1-\bar{x}_2)}{\sqrt{S.E._1{}^2+S.E._2{}^2}},$$

when $\bar{x}$ is the mean, and S. E. is the standard error of the mean.

RESULTS OF DEVELOPMENT STUDIES

EGG

The eggs are usually laid in groups of 3 or 4 in the cork supplied for oviposition. Frequently, however, as many as 20 to 30 were found in a cluster.

From inspection of the data in table 1, it is seen that atmospheric moisture has little or no effect on the rate of development of the egg. The length of the egg stage varies considerably with the temperature. Eggs failed to develop at 15° or at 40° C. At 20° somewhat more than 12 days were required for them to hatch, while the greatest rate of development occurred at 35° when only a little over 4 and a fraction days were necessary. Although separate series of data are given for each of the three foods, all are computed on a similar basis and a comparison of the results will give an idea of the reliability of the differences caused by temperature changes.

TABLE 1.—*The development of the saw-toothed grain beetle under various conditions of temperature, moisture, and food*

Food and temperature (° C.)	Saturation deficit	Relative humidity	Egg		Larva			Pupa		Total		
			N	$\bar{x}$	*N*	$\bar{x}$	Standard error	*N*	$\bar{x}$	*N*	$\bar{x}$	Standard error
Rolled oats:	*Millimeters*	*Percent*		*Days*		*Days*			*Days*		*Days*	
20°	5.0	71.6	19	12.79	19	39.74	0.66	18	16.33	18	69.06	0.90
	12.5	28.7	36	11.58	36	44.64	.85	14	17.43	14	73.64	1.02
25°	5.0	78.8	45	6.29	45	15.51	.27	45	8.53	45	30.31	.23
	12.5	47.3	32	6.75	32	17.66	.48	31	8.13	31	32.23	.43
	22.5	5.2	44	6.84	44	23.61	.37	40	8.55	40	39.15	.43
30°	5.0	84.3	36	4.89	36	10.28	.19	36	5.50	36	20.67	.17
	12.5	60.7	36	4.99	36	12.28	.31	36	5.51	35	22.80	.30
	22.5	29.2	36	5.00	36	15.72	.25	34	5.74	34	26.41	.27
35°	5.0	88.1	43	4.16	43	9.37	.14	43	4.47	43	18.00	.14
	12.5	70.4	33	4.30	33	10.85	.45	33	4.67	33	19.82	.44
	22.5	46.7	45	4.31	45	14.84	.25	45	4.71	45	23.87	.30
English walnuts:												
20°	5.0	71.6	31	12.61	31	44.26	.74	17	15.12	17	70.59	1.00
	12.5	28.7	19	12.32	19	52.47	.86	3	16.00	3	77.67	1.78
25°	5.0	78.8	44	6.52	44	18.32	.29	40	7.83	40	32.73	.33
	12.5	47.3	31	6.65	31	21.74	.52	25	8.32	25	36.52	.66
	22.5	5.2	50	6.70	50	33.08	.77	31	9.29	31	48.55	1.25
30°	5.0	84.3	29	5.03	29	13.59	.36	28	5.68	28	24.11	.43
	12.5	60.7	31	5.03	31	15.74	.31	28	5.93	28	26.54	.37
	22.5	29.2	50	4.98	50	21.20	.46	30	5.47	30	30.97	.52
35°	5.0	88.1	37	4.00	37	15.30	.29	22	4.45	22	23.68	.45
	12.5	70.4	32	4.09	32	16.09	.47	14	4.79	14	25.14	.71
	22.5	46.7	29	4.24	29	21.76	.96	14	4.57	14	29.57	.89
Raisins:												
20°	5.0	71.6	3	12.00	3	51.00	-------	3	17.67	3	80.67	-------
25°	5.0	78.8	10	6.20	10	30.10	-------	5	8.20	5	40.80	-------
	12.5	47.3	10	6.20	10	32.50	-------	10	8.40	10	47.20	-------
30°	5.0	84.3	4	4.00	4	21.50	-------	1	6.00	1	36.00	-------
	12.5	60.7	8	4.00	8	27.50	-------	8	5.63	8	36.63	-------

LARVA

Since the eggs failed to hatch at 15° C., some were hatched at 20°, the next higher point in the temperature series, and the newly hatched

larvae were placed on rolled oats at a saturation deficiency of 5 mm. and a temperature of 15°. The larvae failed to develop. Under the same food and atmospheric moisture conditions at 20°, larvae developed in an average of 39.74 days as compared with 15.51 days at 25°. On rolled oats the optimum temperature for development is about 35° where 9.37 days are required for the larval stage, while at 30° the developmental time is 10.28 days. With walnuts as food, and under the same moisture conditions, the developmental optimum appears nearer to 30°, where 13.59 days are required as compared with 15.30 days at 35° C.

In general the data show significant differences in the rate of larval development at the three moisture levels within each temperature. Larval development tends to be more rapid at a higher humidity. The differences in developmental time between the moisture levels are greatest at 20° C. on both rolled oats and walnuts, because of the longer periods involved; in other words, differences in the developmental period decrease with an increase in temperature. Between 12.5 mm. and 22.5 mm. of saturation deficiency the data differ more because of the greater spread in atmospheric moisture conditions. Because of insufficient numbers, the raisin data are not statistically treated. From inspection, however, they appear to show the same trend as the other foods.

On the basis of the rate of development, rolled oats is superior to either walnuts or raisins as food for the saw-toothed grain beetle. The raisin data are admittedly lacking in sufficient replications and are presented only as an indication of the value of raisins as food for this insect. Comparing the rates of development at 25° C. and 5-mm. saturation deficiency, the larvae completed development in 15.51 days on rolled oats, 18.32 days on walnuts, and 30.1 days on raisins.

PUPA

The rate of development of the pupa varies with the temperature, the maximum being 16 to 17 days at 20° C. and the minimum about 4.5 days at 35°. From inspection of the data, the atmospheric moisture or type of food shows little or no effect on the rate of development, although here, as in the egg stage, any actual differences would be slight and would be obscured by the short developmental period and the relatively long intervals between observations. The data, however, are mostly rather uniform within each temperature regardless of humidity, and the values in each class on the different foods approach each other.

TOTAL DEVELOPMENTAL PERIOD

Since food and atmospheric moisture conditions have little or no measurable effect on the rate of development of the egg and pupa, the rate of development under the various conditions for the entire period from egg to adult is similar to that of the larva. On rolled oats at a 5-mm. saturation deficiency, the insect completes its development in the shortest time of 18.0 days at 35° C., while the longest mean period occurs at 20° where 69.06 days are required. With walnuts as food the data for total development at 30° are not significantly different from those at 35°. The optimum condition lies somewhere between these temperatures. The shortest mean period on walnuts at a

5-mm. saturation deficiency is 23.68 days at 35°, while the maximum, which occurs at 20°, is 70.59 days. On raisins the developmental periods appear from the few available data to be somewhat longer. At 30° and at the high humidity, one complete life cycle requires about 36.0 days, while at 20° the complete developmental time is 80.67 days.

The longest total period of individual development recorded in these experiments was 84 days on raisins at 20° C. and a 5-mm. saturation deficit. Under the same conditions, but on rolled oats instead of raisins, the longest period was 78 days, the shortest 64 days. The comparable data for 35° with rolled oats as the food are 20 and 17 days, respectively.

DISCUSSION

Chapman and Baird (*5*) determined the duration of the developmental stages of *Tribolium confusum* Duval at several temperatures

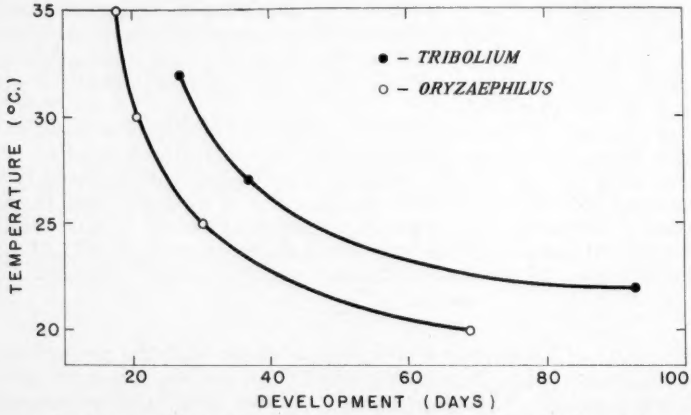

FIGURE 1.—Comparison of the total developmental periods at different temperatures for *Oryzaephilus surinamensis* fed on rolled oats at 5 mm. saturation deficiency and *Tribolium confusum* fed on whole-wheat flour at 75 percent relative humidity.

and 75 percent relative humidity. In figure 1 are shown these data in comparison with the present determinations for *Oryzaephilus*. The data, although not exactly comparable, are nearly so. In the second species the data plotted are those at 5 mm. saturation deficiency, but the latter is close enough to 75 percent relative humidity that not over 1½ days correction would be necessary for developmental periods of 70 days or more. Furthermore, *Oryzaephilus* was reared on rolled oats whereas *Tribolium* had whole-wheat flour as food. These foods, however, are not widely different. The comparison of the two species with respect to the time required for development from egg to adult shows *Oryzaephilus* to be considerably faster than *Tribolium confusum* in its development. At 30° C. the difference amounts to about 8 days; at 22°, to about 52 days. Therefore the development of *Tribolium confusum* under somewhat comparable conditions may take over twice the time required by *Oryzaephilus*.

Holdaway (*9*) studied the development of *Tribolium confusum* at several relative humidities and a single temperature (27° C.) in connection with population studies. He found a 24.4-percent increase in the total developmental period when the relative humidity was reduced from 75 to 25 percent. In the present studies with *Oryzaephilus* at 25° a comparable lengthening of the period was found amounting to a 25.9-percent increase when the saturation deficit was increased from 5.0 to 22.5 mm. pressure (a drop from 78.8 to 5.2 percent relative humidity).

The fact that stored-product insects are able to develop in very dry food is well known. Certain insects can gain water from an atmosphere which is nearly saturated, the water entering through channels other than the mouth. Babcock (*2*) pointed out that through oxidation of food material a considerable quantity of water is produced which many animals use to carry away nitrogenous wastes in solution. Insects living on dry foodstuffs, however, excrete solid uric acid and are able to retain the water of metabolism which would otherwise be used in excretion.

The water content of their food has been shown to influence the proportion of water in insects. Insects that feed on food of high moisture content contain a much higher percentage of water than those that live on dry materials. Living on dry food, the insects themselves contain a higher percentage of water than there is in the food. Schultz (*13*) has shown that larvae of *Tenebrio molitor* L. fed on bran pass feces of which 80 percent is undigested food but lower in moisture than the original bran. Buxton (*4*) explained this as the result of wasteful eating as well as the formation of water of metabolism. When food is eaten wastefully the small amount of free water contained in it is probably extracted by mechanisms in the rectum, which results in dry excreta. Schwardt (*14*) observed the saw-toothed grain beetle develop in food containing practically no free water.

According to Buxton (*4*) starved larvae of *Tenebrio molitor* lose weight when exposed to a humidity of 80 percent or lower, but if kept at 90 percent and temperatures of 23° or 30° C. they gain weight. He stated that the phenomenon is not similar to the hygroscopy of hair and other dead materials, but that it is due to biological activity. Water from the liquid in the tracheoles is thought to be continuously secreted into the insect body, thereby increasing the concentration of the tracheole liquid and causing atmospheric moisture to be absorbed by it.

Stored-product insects are able to conserve moisture in various ways, thus protecting themselves against desiccation. Mellanby (*10*) stated that practically all the water that evaporates from an insect is lost through the spiracles. When the spiracles are closed the air within the tracheae becomes saturated and in most cases remains so when the spiracles are opened. Otherwise, when the air becomes very dry, the air of the tracheae becomes dry, the tissues being unable to supply water fast enough. One of the best methods that insects possess for protecting themselves against loss of water, then, is their ability to close the spiracles. Certain factors, however, such as an increase in temperature, may accelerate metabolic activity and increase the rate of respiration, causing the spiracles to be opened more often and increasing the rate of water loss.

The effect of atmospheric moisture on a stored-product insect,

therefore, is in proportion to the rate at which moisture is being withdrawn from the insect and to the ease with which that moisture can be replaced. The longer time required for development on walnuts than on oats is probably due in considerable part to the lower hygroscopicity of the food.

LETHAL EFFECTS OF HIGH AND LOW TEMPERATURES

The lethal effects of high and low temperatures on insects are known to vary with the species, the developmental stage, and the other environmental factors, such as moisture. Data have been presented in the preceding paragraphs showing that the egg and larva of the saw-toothed grain beetle are incapable of developing at 40° and 15° C. To obtain further information on the reactions of this insect to lethal temperatures, its longevity was determined near both the upper and the lower limits of development. Only healthy adult insects, of which the sex and age were disregarded, were used in these experiments, since it was not feasible at the time to add greatly to the experimental work by determining the effects on all stages.

Oosthuizen (*12*), working with all stages of the confused flour beetle, *Tribolium confusum*, found some variation in the heat resistance of different stages at different temperatures. He showed that at 44° C. 50 percent of the adult beetles were killed in 7.4 hours and that changes in the atmospheric moisture conditions had no effect at this temperature. At 46° only 64 minutes were required to kill 50 percent of the adults, but at this temperature moisture conditions affected the results, the beetles dying much sooner at 100 percent relative humidity and somewhat sooner at 0 and 30 percent than at 75 percent.

Temperatures between 44° and 46° C. are critical, and very slight changes in the intensity of heat between these points produce profound effects. The writers operated the same apparatus used by Oosthuizen, but it is felt that somewhat greater accuracy in measurement and control of the bath temperatures was attained. The temperatures recorded by Oosthuizen as 44° and 46° are actually slightly lower, although certainly not over 0.7° lower, and probably about half that much.

Adult beetles were exposed in a water bath arrangement and with a technique essentially the same as that described by Oosthuizen. Cages made of a fine-mesh wire screen were used instead of glass and bolting cloth. Thermometers were not placed in the cages as it was found that the desired temperature could be obtained there by maintaining the outside water bath temperature 0.7° C. higher. This difference was due to loss of heat from the exposed portions of each glass exposure chamber at the water surface. Temperatures were measured with a sensitive thermometer graduated in tenths of a degree centigrade. Cage temperatures did not vary more than $\pm 0.1°$. The exposure chambers were conditioned for at least 4 hours prior to each experiment. As active saw-toothed grain beetles cannot live long without food, a small amount of food was provided in each cage. Exposed beetles were allowed 2 days for recovery before the percentage of mortality was determined. Each point plotted in figure 2 represents in excess of 150 individuals.

Tattersfield and Morris (*15*) have shown that mortality data can best be compared at the point at which 50 percent of the individuals

survive. The writers' data for adults of *Tribolium confusum* show somewhat lower values than those of Oosthuizen. At 44° C. and 30 percent relative humidity, 50 percent of the beetles were killed in a little over 4 hours while at 46° and 50 percent relative humidity about 40 minutes were required, as compared with 7.4 hours and 60 to 75 minutes respectively as found by Oosthuizen. These differences show how important a fraction of a degree may be in shifting the time required to kill a group of insects.

The exposure periods necessary to kill 50 percent of the adults of the saw-toothed grain beetle decrease rapidly with slight increases in heat intensity between the temperatures tested. At 42° C. 50-percent mortality occurs in about 34 hours while at 44°, only 2° higher,

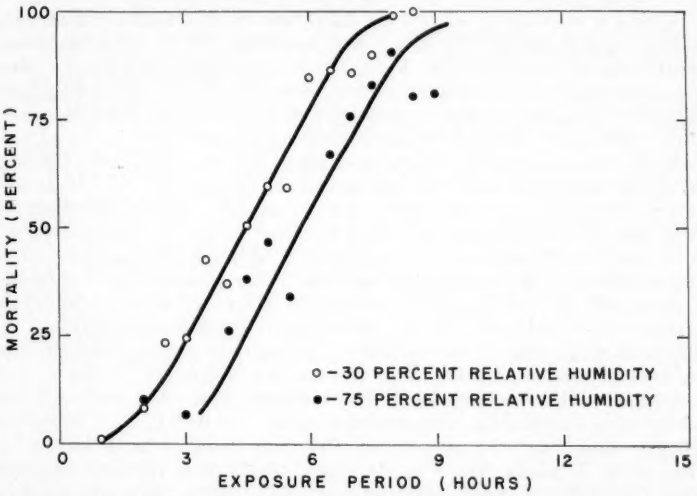

FIGURE 2.—Mortality of the saw-toothed grain beetle exposed for different lengths of time at 44° C. and relative humidities of 30 and 75 percent.

50 percent of the beetles are killed in approximately 4.5 hours at 30 percent relative humidity, and 5.7 hours at 75 percent relative humidity (fig. 2).

In general the saw-toothed grain beetle and the confused flour beetle are about equally resistant to high temperature. The chief difference between these species appears to lie in the higher sensitivity of *Oryzaephilus* to desiccation. At 44° C. this species is definitely more easily killed at 30 percent relative humidity then at 75 percent. At the same temperature and humidities, *Tribolium confusum* is unaffected by the moisture differences. It appears likely that this fact is correlated with the greater susceptibility of *Oryzaephilus* to desiccation at ordinary temperatures.

Nagel and Shepard (*11*) studied the effects of low temperatures on all stages of *Tribolium confusum*. The exposure periods necessary to obtain a 50-percent mortality of the adults at 7°, −6°, −12°, and −18° C. were 336, 8.4, 0.23, and 0.15 hours respectively.

Adult saw-toothed grain beetles were exposed in small fine-mesh wire screen cages, each cage containing approximately 50 beetles and

a small quantity of food material. The cages were placed in dry desiccators in refrigerated cabinets at the desired temperatures. No attempt was made to control the atmospheric moisture conditions, but the temperatures were maintained to within ±1.0° C. Two days were allowed for the insects to recover before mortality was determined. Exposures were made at two temperatures, 10° and 2°. The exposure periods to obtain 50-percent mortality were 30 days and 105 hours respectively. It is impossible from these determinations to say whether *Oryzaephilus* differs significantly from *T. confusum* in resistance to low temperature.

SUMMARY

A study was made of the development of all stages of the saw-toothed grain beetle (*Oryzaephilus surinamensis* L.) under various conditions of temperature, atmospheric moisture, and food. Rearings were made or attempted at temperatures of 15°, 20°, 25°, 30, 35°, and 40° C., at saturation deficiencies of 5, 12.5, and 22.5 mm., and with rolled oats, English walnuts, and raisins as food.

At 5 mm. saturation deficit and on rolled oats as food, the total life cycle from egg to adult requires 69.06 days at 20° C., 30.31 days at 25°, 20.67 days at 30°, and 18 days at 35°. With rolled oats as food, the developmental optimum appears at 35° C. while with walnuts, the developmental optimum occurs between 30° and 35°. Eggs larvae failed to develop at 15° and 40°. In general, development is more rapid at the higher humidities. The egg and pupal stages appear to be little affected by atmospheric moisture conditions. On the basis of the rate of development, rolled oats are superior to either walnuts or raisins as a food for the saw-toothed grain beetle.

Adults of the saw-toothed grain beetle and the confused flour beetle were exposed to high temperatures. At 44° C. and 30-percent relative humidity, 50 percent of the flour beetles were killed in a little over 4 hours while at 46° and 50-percent relative humidity about 40 minutes were required. At 42°, 50 percent of the adult saw-toothed grain beetles were killed in 34 hours while at 44° the exposure periods were approximately 4.5 and 5.7 hours at 30- and 75-percent relative humidities respectively.

Adult saw-toothed grain beetles exposed at 10° and 2° C. gave 50-percent mortality values at 30 days and 105 hours respectively.

LITERATURE CITED

(1) Atwater, W. O., and Bryant, A. P.
1906. The chemical composition of American food materials. U. S. Off. Expt. Stas. Bul. 28, 87 pp., illus. (Revised.)

(2) Babcock, S. M.
1912. Metabolic water: its production and role in vital phenomena. Wis. Agr. Expt. Sta. Res. Bul. 22, pp. [87]–181.

(3) Back, E. A., and Cotton, R. T.
1926. Biology of the saw-toothed grain beetle, Oryzaephilus surinamensis Linné. Jour. Agr. Res. 33: 435–452, illus.

(4) Buxton, Patrick A.
1932. Terrestrial insects and the humidity of the environment Biol. Rev. Cambridge Phil. Soc. 7: [275]–320, illus.

(5) Chapman, Royal N., and Baird, Lilian.
1934. The biotic constants of Tribolium confusum Duval. Jour. Expt. Zool. 68: 293–304, illus.

(6) CHITTENDEN, F. H.
1896. INSECTS AFFECTING CEREALS AND OTHER DRY VEGETABLE FOODS. U. S. Dept. Agr., Div. Ent. Bul. 4 (n. s.): 112–130, illus.
(7) FISHER, R. A.
1938. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 7, rev. and enl., 356 pp., illus. Edinburgh and London.
(8) HAYDAK, MYKOLA H.
1936. A FOOD FOR REARING LABORATORY INSECTS. (Sci. Note) Jour. Econ. Ent. 29: 1026.
(9) HOLDAWAY, F. G.
1932. AN EXPERIMENTAL STUDY OF THE GROWTH OF POPULATIONS OF THE "FLOUR BEETLE" TRIBOLIUM CONFUSUM DUVAL, AS AFFECTED BY ATMOSPHERIC MOISTURE. Ecol. Monog. 2: 261–304, illus.
(10) MELLANBY, KENNETH.
1935. THE EVAPORATION OF WATER FROM INSECTS. Biol. Rev. Cambridge Phil. Soc. 10: 317–333, illus.
(11) NAGEL, R. H., and SHEPARD, H. H.
1934. THE LETHAL EFFECT OF LOW TEMPERATURES ON THE VARIOUS STAGES OF THE CONFUSED FLOUR BEETLE. Jour. Agr. Res. 48: 1009–1016, illus.
(12) OOSTHUIZEN, M. J.
1935. THE EFFECT OF HIGH TEMPERATURE ON THE CONFUSED FLOUR BEETLE. Minn. Agr. Expt. Sta. Tech. Bul. 107, 44 pp., illus.
(13) SCHULTZ, FR. N.
1930. ZUR BIOLOGIE DES MEHLWURMS (TENEBRIO MOLITOR). I. MITTEILUNG: DER WASSERHAUSHALT. Biochem. Ztschr. 227: 340–353.
(14) SCHWARDT, H. H.
1934. THE SAW-TOOTHED GRAIN BEETLE AS A RICE-MILL PEST. Ark. Agr. Expt. Sta. Bul. 309, 14 pp.
(15) TATTERSFIELD, F., and MORRIS, H. M.
1924. AN APPARATUS FOR TESTING THE TOXIC VALUES OF CONTACT INSECTICIDES UNDER CONTROLLED CONDITIONS. Bul. Ent. Res. 14: 223–233, illus.
(16) WILSON, ROBERT E.
1921. HUMIDITY CONTROL BY MEANS OF SULFURIC ACID SOLUTIONS, WITH CRITICAL COMPILATION OF VAPOR PRESSURE DATA. Jour. Indus. and Engin. Chem. 13: 326–331, illus.

RETENTION BY SOILS OF THE NITROGEN OF VARIOUS COMPOUNDS AS SHOWN BY SUBSEQUENT PLANT RESPONSE [1]

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INTRODUCTION

Although nitrates and nitrites dissolved in the soil solution are not held by the solid phase of the soil against leaching, the ammonia form of nitrogen is strongly retained. Soil chemists have known these facts for many decades. The reactions of many other nitrogenous compounds with soils, however, have been but little studied. Do soil solids generally fail to retain the nitrogen contained in all of the more or less acidic anions like nitrate and nitrite? On the other hand, is the nitrogen in all the cations which exhibit only moderately basic properties retained by the soil solids as is that in ammonium ions? Experiments to test these questions further were undertaken in this study. Regardless of how accurately the reactions of the soil with moderately acidic or basic ions may be foretold, questions regarding the retention of nitrogen-carrying units of intermediate or amphoteric properties are not answered thereby; nitrogenous compounds with neither pronounced anionic nor cationic properties were accordingly included in this study.

With but few exceptions, soil-fertility investigations directed toward ultimate application to field conditions must encounter the factor of percolating waters resulting from natural rainfall or irrigation. Soluble nitrogen-containing units, whether in ionic form or not, may be dissolved in the irrigation water and applied to the soil in this way. They may also arise in the soil either through the solution of nitrogenous fertilizers previously applied in a dry state, or as the end products of purely chemical reactions or of biological activity. In this connection, the soluble intermediate products of such transformations cannot be neglected. With such an important element of fertility as nitrogen, knowledge of the reactions with the soil of the various units carrying this element is important for efficient progress on many problems.

METHODS

The method described by Conrad and Adams (*3*)[2] was used because it not only gives a semiquantitative estimate of the reaction of the compound in question with the soil, but also indicates the effect of the compound on the growth of the test plants. In figure 1 is shown a column of pots illustrating the general method used. Each 4-inch pot, previously coated with asphaltum paint, was provided with a square of waxed paper to cover the drainage hole. Usually 400 gm.

[1] Received for publication September 5, 1939. Investigations of the Division of Agronomy, California Agricultural Experiment Station, Davis, Calif.

[2] Italic numbers in parentheses refer to Literature Cited, p. 630.

of dry soil deficient in nitrogen was then added to each pot. The three pots of each column were stacked with the bottom one nesting in the drainage can and with the consecutive pots in the column held apart by a cannery-tin top punched with a large hole. The large 5-inch pot above, equipped with a single-holed rubber stopper, was used as a reservoir for the test solution. A piece of glass tubing drawn out to a long taper and inserted in the hole of the rubber stopper constituted, with the stopper, a needle valve. The rate of flow was then easily regulated by raising or lowering this tapered tubing. Thus the test solution was allowed to drip slowly down upon the column of pots. At the outset, sufficient solution was added to the reservoir to wet the soil completely and to give some drainage through the bottom pot. A somewhat greater excess was used when

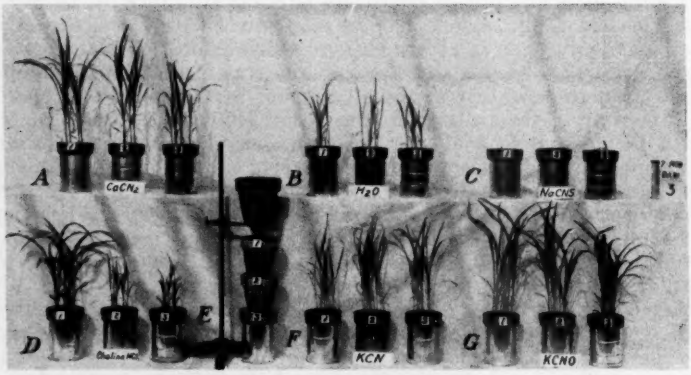

FIGURE 1.—Effect on plants of the retention of nitrogen from various percolating solutions. At *E* the solution being tested slowly dripped from the reservoir above, down upon the column of 4-inch pots (1, 2, and 3), each containing about 400 gm. of dry soil deficient in nitrogen. After percolation the pots in the columns were taken down and subsequently cropped. The test solution used in each case is shown on the card leaning against pot 2 of that column. The soil showed practically no ability to retain nitrogen in the anionic units of KCN (*F*), KCNO (*G*), and NaCNS (*C*). The nitrogen of choline hydrochloride (*D*) was completely retained in the top pot. $CaCN_2$ (*A*) showed intermediate properties.

these leachings were to be analyzed. In general the solutions were percolated through the columns as rapidly as possible. Usually within 12 hours after percolation started, the solution had disappeared from the reservoir above and from the surface of the soil in the top pots, and water had appeared in the drainage can. Except as noted below, no effort was made to prolong the actual percolation. Generally in from 24 to 48 hours after percolation the pots were taken down and cropped, each pot being provided with a separate drainage can. In some tests, the seed had been planted in the dry soil before percolation; in others, planting was deferred until a few days or more after the columns were dismantled. Double Dwarf milo and Double Dwarf darlo, varieties of grain sorghum (*Sorghum vulgare* Pers.) commercially grown in California, were used as test plants.

In experiments reported with phosphorus compounds (*2*), the pots

were weighed before and after percolation to evaluate the uniformity in the amounts of moisture held by the soil in the various pots of the column. These showed variations of less than 10 percent of the moisture present between that held by the top pots and that held by the bottom ones. Even though weighings of pots were only occasionally made for the tests reported herein, the variations in moisture held by the soil were assumed to be no larger than in earlier studies.

Nonretention of the compound or at least its nitrogen-containing units by the solid phase of the soil was indicated by the nearly equal distribution of the nutrient (or toxic) effects of the solute, assuming equal distribution of the solvent among the pots of the column. Unequally enhanced (or in cases of toxicity, injured) growth among the pots of the column, with the divergence from the corresponding pots of the distilled-water columns being greater in the top pot and successively less or absent in lower pots, indicated retention. Nitrogen-containing (or in cases of injury, toxic) units that were retained had reacted either physically or chemically with the soil by becoming more or less firmly affixed to the solid particles of the soil and had thereby been more or less completely removed from the solvent as it trickled by.

The soils used in this study were as follows:

Yolo fine sand (a loamy fine sand), C–11, was secured as a deep subsoil sample from a Yolo soil on the southern bank of Putah Creek on the university farm at Davis. This soil and the next two are described in the Soil Survey report of the Dixon area (*4*).

Yolo silt loam, C–62, was secured as a surface sample from the experimental plots of the Division of Agronomy, at Davis; it had previously been cropped to small grains.

Yolo fine sandy loam, C–68, was secured as a subsoil sample from a depth of 1 to 2 feet from the agronomy experimental area on land that had been planted to a variety of crops and that had lain fallow for a few months before the sample was collected.

Fresno fine sandy loam, C–10, was secured as a surface-soil sample from a vineyard about 10 miles northwest of the city of Fresno. This soil is described in the soil survey of the Middle San Joaquin Valley (*9*).

The nitrogen compounds used in these experiments were as follows:

C. p. chemicals—$Ca(NO_3)_2$, $NaNO_3$, $NaNO_2$, KCN, KCNO, picric acid, NaCNS, $K_4Fe(CN)_6$, $K_3Fe(CN)_6$, NH_4OOCCH_3, $(NH_4)_2CO_3$, NH_4OH, $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$, urea, hydrazine sulfate, and hydroxylamine sulfate.

Chemicals of other grades—acetamide (purified crystals), peptone (bacteriological), egg albumin (impalpable powder, soluble), gelatin (granular edible), blood albumin (technical), commercial sulfamic acid, and ammonium sulfamate; cyanamide ($CaCN_2$) bought as a fertilizer.

Chemicals of highest purity—guanidine carbonate, theobromine, glycine, hippuric acid, glutamic acid, formamide, dicyandiamide, phenylurea, thiourea, cyanuric acid, acetonitrile, and acetoxime, and hydrochlorides of methylamine, dimethylamine, trimethylamine, choline, semicarbazide, cysteine, and arginine.

Chemical of practical grade—tetramethylammonium chloride.

GROWTH STUDIES

The tests with the various nitrogen compounds were conducted as time and the supply of materials permitted. Included in each growth

series were columns percolated with distilled water to serve as checks in evaluating the data secured with the various solutions. The main interest in these data arises from the behavior of different forms of nitrogen on the basis of the chemical properties of each. Since the various growth series could not be arranged according to the chemical properties of the compounds, it seemed best to report the data in table 1 not as the compounds were grouped in the separate growth series but in a recapitulated form arranged as follows: Section A, nitrogen in anionic units; B, nitrogen in cationic units; C, nitrogen in amphoteric or approximately neutral units; and D, checks with distilled water and cultural data applicable to each growth series.

In section A were placed sulfamic and picric acids, both fairly strong acids, as well as the salts of either weak or strong acids. Nitrourea, though somewhat stronger than acetic acid (*13*), is placed in section C for comparison with urea. All fairly strong bases containing nitrogen, as well as the salts of such bases, are in section B. Section C contains the weaker acids and weaker bases, as well as the amphoteric and approximately neutral organic compounds. Cysteine, even though used as the hydrochloride, belongs to this group.

In sections A, B, and C of table 1 are columns headed "Growth series." By referring to the corresponding growth series in section D, the yields resulting from each solution can be compared

TABLE 1.—*Retention of the nitrogen from various percolating solutions by the solids of different nitrogen-deficient soils in columns of three 4-inch pots as shown by the subsequent average yield of replicated cultures of Double Dwarf milo and darlo*

A, NITROGEN IN ANIONIC UNITS

Percolating solution	Growth series (see sec. D below)	Replications	Average green yield per pot			
			Pot 1, top	Pot 2, middle	Pot 3, bottom	Control
		Number	*Grams*	*Grams*	*Grams*	*Grams*
Calcium nitrate, $Ca(NO_3)_2$	1a	3	[12]18.6	[1]14.4	[1]14.8	
Sodium nitrate, $NaNO_3$	1	3	[1]14.7	[1]24.4	[1]21.7	[1]19.2
Sodium nitrite, $NaNO_2$	1	3	[1]14.3	[1]18.5	[1]19.7	[1]15.4
Potassium cyanide, KCN	4	3	[3]2.7	[1]4.0	[1]4.3	[1]4.3
Potassium cyanate, KCNO	5	3	[1]10.3	[1]10.1	[4]8.6	
Sulfamic acid, HSO_3NH_2	3	3	[1].0	[1].0	[1].1	[1].0
Picric acid, $(NO_2)_3C_6H_2OH$	4	3	[1].1	[1].1	[1].3	[1].0
Sodium thiocyanate, NaCNS	4	3	[3].2	[1].1	[3].1	[1].1
Potassium ferrocyanide, $K_4Fe(CN)_6$	4	3	1.2	1.3	1.4	.9
Potassium ferricyanide, $K_3Fe(CN)_6$	4	3	1.4	.9	1.0	1.0
Calcium cyanamide, $CaCN_2$	2	3	[1]8.2	[4]5.3	[1]1.9	1.9

B, NITROGEN IN CATIONIC UNITS

Ammonium acetate, NH_4OOCCH_3	3	3	[12]10.3	1.9	1.7	[1]5.0
Ammonium carbonate, $(NH_4)_2CO_3$	3	3	[18]10.4	1.0	1.0	[1]5.4
Ammonium hydroxide, NH_4OH	1	3	[18]43.4	[4]2.0	1.7	[1]20.3
Ammonium phosphate, $(NH_4)_2HPO_4$	3	3	[18]14.0	1.2	1.2	[1]9.6
Ammonium sulfamate, $NH_4SO_3NH_2$	3	3	[16]4.4	[4].5	[1].3	2.4
Ammonium sulfate, $(NH_4)_2SO_4$	1	3	[16]48.7	1.5	1.2	[1]26.6
Hydrazine sulfate, $N_2H_4H_2SO_4$	4	3	[12]3.1	1.4	1.7	[4]2.5
Hydroxylamine sulfate, $(NH_3OH)_2H_2SO_4$	7	5	[14]7.7	1.9	[3]2.3	[1]3.2
Tetramethylammonium chloride, $(CH_3)_4NCl$	7	3	[18]19.6	1.7	1.8	[1]13.7
Hydrochlorides:						
Trimethylamine, $(CH_3)_3N.HCl$	7	3	[16]21.6	1.9	1.8	[1]12.0
Dimethylamine, $(CH_3)_2NH.HCl$	7	3	[16]21.7	1.6	1.6	[1]13.0
Methylamine, CH_3NH_2HCl	7	3	[1][24.0	1.4	1.5	[1]11.1
Choline, $HO(CH_2)_2N(CH_3)_3OH.HCl$	5	3	[1][22.7	1.6	1.4	
Arginine, $C_6H_{14}N_4O_2.HCl$	7	6	[18]15.8	1.9	1.9	[1]8.1
Semicarbazide, $N_2H_4CONH_2.HCl$	4	3	[12]7.2	[12]2.5	[12]2.5	[1]4.1
Guanidine carbonate, $NH:C(NH_2)_2.H_2CO_3$	6	3	[4]1.0	[15]5.1	4.4	3.1

TABLE 1.—*Retention of the nitrogen from various percolating solutions by the solids of different nitrogen-deficient soils in columns of three 4-inch pots as shown by the subsequent average yield of replicated cultures of Double Dwarf milo and darlo*—Continued

C, NITROGEN IN AMPHOTERIC OR APPROXIMATELY NEUTRAL UNITS

Percolating solution	Growth series (see sec. D below)	Replications	Average green yield per pot			
			Pot 1, top	Pot 2, middle	Pot 3, bottom	Control
		Number	*Grams*	*Grams*	*Grams*	*Grams*
Glycine, NH_2CH_2COOH	3	3	[3] 7.4	[1][2] 8.3	[4] 3.5	[1] 4.1
Do	7	5	[1][5] 15.8	[1][4] 9.2	[1] 2.1	[1] 10.2
Hippuric acid, $C_6H_5CONHCH_2COOH$	6	3	7.4	5.8	4.9	5.0
Glutamic acid, $C_5H_9NO_4$	4	3	[1][6] 17.8	[1][2] 8.6	[4] 8.0	3.0
Cysteine hydrochloride, $HOOCCHNH_2CH_2SH.HCl$	7	5	[1][5] 14.7	[1][5] 4.4	1.6	[1] 10.5
Formamide, $HCONH_2$	3	3	[1][5] 11.4	[1][2] 5.6	1.6	[1] 3.8
Acetamide, CH_3CONH_2	3	3	[3] 5.1	[3] 5.6	[1][6] 6.0	[1] 7.0
Dicyandiamide, $NH_2C(:NH)NHCN$	5	3	1.1	1.0	1.2	
Urea, NH_2CONH_2	3	3	[1][6] 6.9	[1][5] 7.7	3.1	[1][6] 6.8
Do	1a	3	[1][5] 5.5	[1][2] 3.1	1.8	
Phenylurea, $C_6H_5NHCONH_2$	3	3	2.2	3.7	3.2	[1][5] 5.0
Nitrourea, $NH_2CONHNO_2$	4	3	[1][5] 6.1	[1][4] 4.0	[1][4] 4.1	[1][5] 5.8
Thiourea, NH_2CSNH_2	6	3	[4] .04	[1] .04	[1] .2	[1] .4
Cyanuric acid, $H_3C_3N_3O_3$	3	3	1.4	1.4	1.4	1.7
Acetonitrile, CH_3CN	7	5	[1][5] 3.2	[1][6] 4.2	5.2	[1][5] 5.0
Acetoxime, $(CH_3)_2C:NOH$	7	4	[1] 2.9	[1][2] 2.5	[1][2] 2.7	[1] 3.1
Peptone	4	3	[1][2] 13.3	3.7	2.4	[1][5] 5.0
Egg albumin	4	3	[1] 9.8	[1][6] 7.1	[1][2] 4.4	[4] 4.7
Blood alumin	4	3	[4] 5.9	1.3	1.7	2.4
Gelatin	1	3	[1][2] 36.0	1.6	1.4	[1] 14.0
Theobromine, $C_7H_8N_4O_2$	7	5	[1][5] 10.0	1.8	1.8	[1] 2.9

D, CHECKS WITH DISTILLED WATER

Growth Series No.	Experiment began	Experiment ended	Replications	Soil No.	Crop	Average green yield per pot			
						Pot 1, top	Pot 2, middle	Pot 3, bottom	Control
			Number			*Grams*	*Grams*	*Grams*	*Grams*
1	Mar. 19, 1938	Apr. 20, 1938	3	C-11	Milo	1.6	1.6	1.7	1.7
1a	Apr. 10, 1938	May 25, 1938	3	C-11	do				
2	Oct. 11, 1937	Nov. 14, 1938	3	C-62	do	1.0	.9	.9	1.4
3	Nov. 9, 1938	Dec. 16, 1938	3	C-68	Darlo	1.4	1.4	1.6	1.4
4	Sept. 17, 1938	Nov. 9, 1938	3	C-68	do	1.4	1.2	1.4	1.7
5	Sept. 28, 1938	Nov. 16, 1938	1	C-68	do	.6	.4	1.1	
6	July 16, 1938	Aug. 15, 1938	3	C-10	do	5.0	3.3	4.4	4.5
7	Mar. 20, 1939	Apr. 17, 1939	5	C-68	Milo	2.1	1.8	1.8	1.5

[1] Statistically different (*5, p. 114*) from the corresponding pots of the distilled-water columns as reported in sec. D. P=0.01 or less.
[2] Statistically different (*5, p. 112*) from the value in the column to the right and for the pot next below it in the column of pots. P lies between 0.02 and 0.05.
[3] Statistically different (*5, p. 114*) from the corresponding pots of the distilled-water columns as reported in sec. D. P lies between 0.01 and 0.02.
[4] Statistically different (*5, p. 114*) from the corresponding pots of the distilled-water columns as reported in sec. D. P lies between 0.02 and 0.05.
[5] Statistically different (*5, p. 112*) from the value in the column to the right and for the pot next below it in the column of pots. P=0.01 or less.
[6] Statistically different (*5, p. 112*) from the value in the column to the right and for the pot next below it in the column of pots. P lies between 0.01 and 0.02.

with the yields similarly and simultaneously secured by using distilled water as the test solution. Section D reports the cultural data applicable to each growth series, such as the time of the experiment, the soil used, and the crop planted. Every reasonable effort was made to keep growth conditions uniform for all cultures in each series. After growth had started and stands had been secured, the pots in

each series were randomized, and the customary care was given. Tap water was used for watering the crops.

The growth series numbered 1a as reported in table 1 inadvertently lacked columns percolated with distilled water. The same soil was used as in series 1, and the growth periods overlapped. No great error is involved, the writer believes, in using the yield figures for distilled-water columns from series 1 to evaluate the results secured for $Ca(NO_3)_2$ and for urea.

In the various growth series, the amount of nitrogen applied per column varied somewhat. In growth series 1, each column received 30 milligram-atoms of nitrogen; in series 1a, 12.4; in series 2, 30 and in each series from 3 to 7 inclusive, 10 milligram-atoms of nitrogen per column.

Besides the yields of the different pots in the columns resulting from the different solutions, table 1 reports yields from control pots grown simultaneously. Each of these received one-third the volume of the same concentration of the test solution in question as was added to the respective column of three pots. The various levels of statistical significance for each of the values are expressed as superscripts given in footnotes.

CHEMICAL TESTS

Some of the growth studies reported in table 1 gave inconclusive evidence with certain compounds. During the revision of the manuscript, it seemed desirable to make a limited number of tests (by methods employed in other studies) with some of the more interesting solutions. Each solution to be tested was added to a single pot containing 400 gm. of dry soil (C–68) in four successive portions applied about 2 hours apart. The first portion, 250 ml., gave about 90 ml. of percolate. The other three portions were of 115 ml. each. Suitable aliquots of the original solution and successive percolates were analyzed for nitrogen by the Kjeldahl method and the concentration of nitrogen in milligram-atoms per liter calculated. The results are shown in table 2.

TABLE 2.—*Concentration of nitrogen as determined by the Kjeldahl method in the original solution and in four successive percolates of approximately 100 ml. each passing through Yolo fine sandy loam*

Solution	Concentration of nitrogen per liter in—				
	Original solution	Percolate 1	Percolate 2	Percolate 3	Percolate 4
	Milligram-atoms	*Milligram-atoms*	*Milligram-atoms*	*Milligram-atoms*	*Milligram-atoms*
Distilled water	0.0	0.8	0.4	0.4	0.2
Guanidine carbonate	26.0	1.2	.2	.4	.4
Cyanuric acid	19.4	13.2	18.2	19.0	19.2
Dicyandiamide	19.4	13.8	18.8	----	20.4
Egg albumin	17.0	1.2	1.4	3.2	5.0
Do	46.2	1.8	5.4	12.6	19.6

NITROGEN IN ANIONIC UNITS

The soils in this study that were stimulated in yield of plants by $Ca(NO_3)_2$, $NaNO_3$, $NaNO_2$, KCN (fig. 1, *F*), and KCNO (fig. 1, *G*)

did not retain the nitrogen of any of these compounds sufficiently to affect significantly the yields of the subsequent crops of sorghum. Sulfamic acid, picric acid, and NaCNS, being toxic, significantly decreased the yields of these crops and were not retained significantly by these soils. The yellow color of the picric acid solution was easily recognized in the leachings from the bottom pot. Neither potassium ferrocyanide nor ferricyanide significantly affected the yields of sorghums in these studies. Judging from the yellow color of the leachings, the soil did not retain the whole of either of these compounds. As is widely recognized, calcium cyanamide rapidly hydrolyzes in the soil to give urea, which in turn is subject to further transformations. Perhaps because of the small number of replicates used, statistically significant differences in yield were not secured between successive pots of the $CaCN_2$ columns (fig. 1, *A*). The yields of the top pots are, however, significantly (*P* lies between 0.02 and 0.05) greater than those of the bottom ones. This is the same type of behavior secured with urea as reported in section C, growth series 3.

NITROGEN IN CATIONIC UNITS

In section B of table 1 are reported the results with solutions containing nitrogen in cationic units. Practically without exception, all the nitrogen present in these units was retained in the top pots of their respective series. With NH_4OH, the increases in the second pots just reached the lowest level of statistical significance. Possibly some NH_3 gas from NH_4OH diffused ahead of the percolating solution. The ammonium nitrogen of all other compounds tested was retained in their respective top pots.

These cultures demonstrate in plant growth what Way, as cited by Russell (*12, p. 148*), showed chemically in 1850. The results with ammonium sulfamate show the beneficial effects of the ammonium ions retained in the top pot superimposed on the toxic effects of the sulfamate ions, which, though not retained by the soil solids, were (sulfamic acid, sec. A) generally present throughout the column in the soil solution.

Since hydrazine as listed by Hodgman (*8*) is a base not much weaker than ammonia, its retention by the soil would be expected. Hydroxylamine, according to Ölander (*11*), has a dissociation constant of 1.07×10^{-8} as a base; evidently it is somewhat weaker than hydrazine, though not sufficiently so to be classed as very weak. Except for semicarbazide, it is probably the weakest base listed in section B.

Results similar to those with ammonium salts were secured with the various methyl-substituted ammonium compounds; the hydrochlorides of methyalmine, dimethylamine, and trimethylamine; and tetramethylammonium chloride. Cultures of some of these are shown in figure 2. The nitrogen of choline, a basic compound occurring in some plants and resulting from the hydrolysis of the lecithins, was retained in the top pot.

Arginine, generally classed as a diamino-monocarboxylic amino acid, has moderately strong basic properties. In consequence, its complete retention in the top pot of the column is not surprising.

Guanidine, a monoacidic base, is almost as strong as sodium hydroxide (*13*). We should, therefore expect it to be retained completely in the top pot of the column. Since there was no growth in the top pot of the column, evidently the guanidine, which is toxic, had been retained. Data in table 2 likewise confirm this retention. In addition, apparently, some transformation products, water-soluble and nitrogen-containing, were formed during percolation and were carried down in the percolating waters, causing significantly

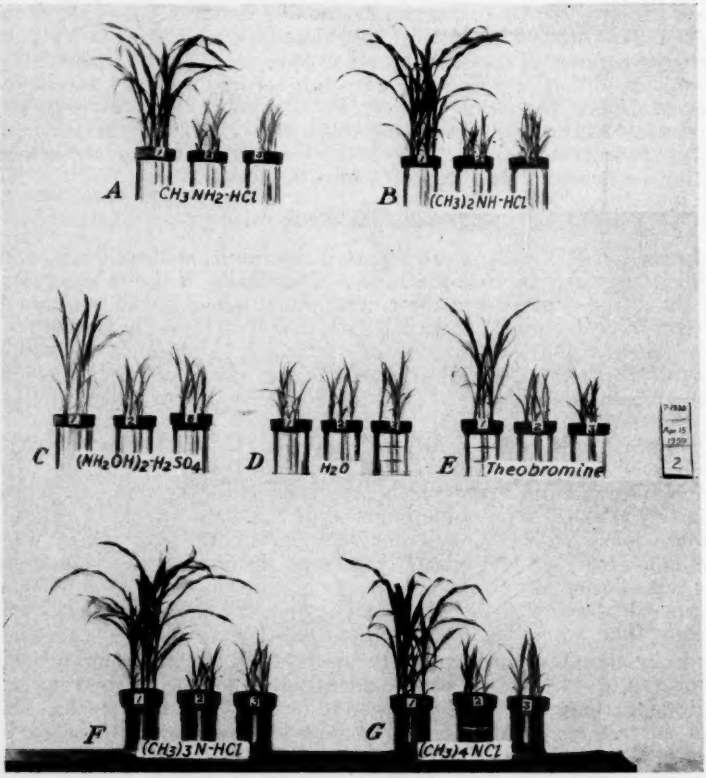

FIGURE 2.—Effect on plants of the complete retention of nitrogen from percolating solutions of various compounds by the soil in the top pots of the respective columns: *A*, *B*, *F*, and *G*, Methyl-substituted ammonium compounds; *C*, hydroxylamine sulfate, *D*, distilled water; *E*, theobromine.

increased growth in the second pot. Semicarbazide (*8*) is a weak base; most of its nitrogen was retained in the top pot. The significantly increased growth of the second and third pots over those of the check would indicate, however, either an incomplete retention of semicarbazide or the movement of some of its decomposition products down in the column.

If a dilute solution of NH_4Cl is shaken up with a portion of soil and then filtered off, and successively shaken up and filtered off, each time with a new portion of the same soil, the concentration of NH_4 ions rapidly diminishes with each successive transfer, until it has practically disappeared from solution. The NH_4 ions have replaced an equivalent amount of other cations such as Ca, Mg, Na, and K and have thereby become attached to the solid phase of the soil. If the amount of solution and the soil be reduced to very small but still finite quantities with the concentrations and ratios remaining constant, the same type of results would be expected.

The layers of soil in the percolating column in order from top to bottom may be regarded as successive portions of the same soil discussed above, arranged naturally so that the filtrate from one automatically comes in contact with and percolates through the one below it by the action of gravity. As the front of the percolating solution of NH_4Cl advances downward through the soil, it may be considered to approach equilibrium more or less completely with each layer in turn. Even if equilibrium is by no means completely attained, still each successive layer of soil will exchange Ca, Mg, Na, and K ions for the NH_4 ions from the percolating liquid as it trickles by. Each successive addition of solution upon passing down through the soil will be higher in concentration of NH_4 ions than its predecessor; hence its original supply of NH_4 ions will penetrate farther down in the soil column. The exchange capacity of the average soil for NH_4 ions is so great and the speed of reaction so rapid, however, that no significant quantities of NH_4 ions would ordinarily leave the top pot of a three-pot column.

NITROGEN IN AMPHOTERIC OR APPROXIMATELY NEUTRAL UNITS

A soil containing 150 milliequivalents of replaceable cations per kilogram may be considered to be fairly well buffered against material change in reaction from solutions of amino acids containing 20 milligram-atoms of nitrogen per liter, especially when the amounts of the solutions are but little more than enough to wet the soil thoroughly. The reaction of the soil through which the solution of the amino acid is percolated is one of the dominating factors in determining the possible chemical reactions of these amphoteric substances. The soils used in these studies were approximately neutral to slightly alkaline.

An amino acid such as arginine with a basic isoelectric point (pI[3] about 10.8) would be expected to dissociate, so that in a neutral soil (about pH 7) its nitrogen-containing ions would be cations. The complete retention of the nitrogen of arginine in the top pot as reported in section B, table 1, was therefore not surprising. A dicarboxylic monoamino acid such as glutamic acid (pI[3] about 3.2) in a neutral soil would be expected to have its nitrogen in anionic units. Though its nitrogen penetrated to a considerable extent into the middle and bottom pots, yet penetration was not the same as with the nitrogen of other anionic units, such as nitrate, nitrite, cyanate, sulfamate, etc. What other properties of glutamic acid are responsible for a greater retention of its nitrogen in the top pot remains to be determined.

[3] pI is the pH of the isoelectric point.

Glycine (pI about 6.0) and cysteine (pI about 5.1) with isoelectric points lying in between those of arginine and glutamic acid were also between them in the ability of the soil to retain the nitrogen of each. Cultures showing the results of tests with some of these compounds appear in figure 3.

Though the growth response in table 1, section C, showed neither benefit nor injury either from cyanuric acid or from dicyandiamide, the chemical data of table 2 clearly indicate that only a very little of the nitrogen of these compounds was retained by the soil.

The nitrogen of formamide was retained rather strongly (table 1, sec. C), but still incompletely by the soil of the top pot. Under the conditions of these experiments, there is no evidence of retention of

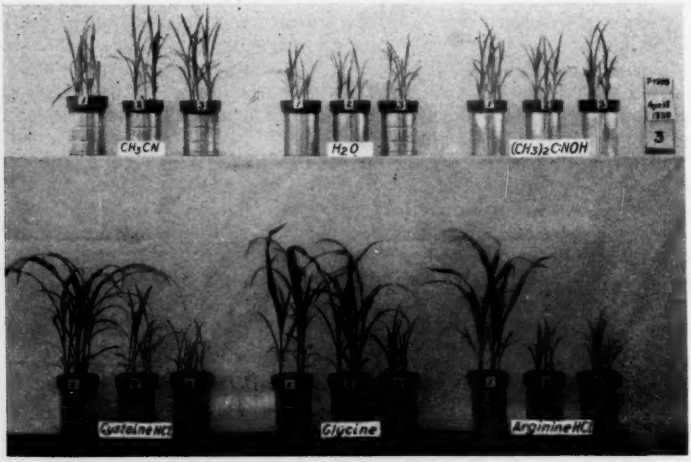

FIGURE 3.—Types of plant response to and retenton by soil of various nitrogenous organic compounds when used in percolating solutions. No retention with acetonitrile (CH_3CN); complete retention with arginine hydrochloride. Intermediate types of retention with the amino acids glycine and cysteine. Acetoxime ($(CH_3)_2C:NOH$, gave but little response and no evidence of retention.

the nitrogen of acetamide, the yields from the three pots of the column being nearly equal (table 1, sec. C). Conant (*1*) regards amides as essentially neutral substances, while Sidgwick (*13*) regards them as very weak bases. Hodgman (*8*) lists acetamide as a base, with, however, a dissociation constant less than that of water. The equivalent conductance as determined (*10, v. 6, pp. 261–262*) at about the same concentrations is slightly more than 10 times as great for acetamide as for formamide. Assuming from these data that formamide is a weaker base than acetamide, the difference in retention by the soil solids between these two amides could not well be explained on the basis of their basic and acidic properties.

For growth series 1a (table 1, sec. C), the trials with urea showed definite and statistically significant but not strong retention of the nitrogen by the soil in the successive pots, beginning at the top. In series 3, although the differences in yields of consecutive pots in the

column are not significant, the yields of the top pots are significantly ($P=0.02$) greater than those of the bottom ones. Some other trials with urea conducted over the last 2 years have suggested the same type of behavior; but insufficient numbers of replicates and high variability in yields have resulted in inadequate statistical significance. It was hoped that the various derivatives of urea tested would show how the substitution of various organic radicals into the molecule affects the retention of urea by the soil, but with the variability encountered, three replications have been insufficient to give even suggestions. Thiourea proved toxic throughout the soil column.

Acetonitrile and acetoxime gave no evidence of being retained by the solids of the soil. With the acetonitrile, in fact, an anomalous situation occurred. A significantly greater yield was secured from the bottom pots than from the middle or top ones.

Peptones, essentially a mixture of polypeptides, might be expected to react as do amino acids in these experiments. The evidence so far gathered and herein reported agrees qualitatively with such an hypothesis. More extended trials will be necessary to determine whether the apparent movement of nitrogen compounds to the second and bottom pots is significant.

The yields of successive pots of the egg-albumin columns are not significantly different. Those of the top pots exceed those of the bottom pots by differences that are highly significant. The chemical tests reported in table 2 clearly show that most of the nitrogen of egg albumin was retained in the top pots. The amounts percolating through the soil, especially in the last percolate, are appreciable, however.

It has been suggested (*3*) that one possible interaction of gelatin with the soil is a filtering out of the dispersed colloid from the percolating solution by the soil as an ultra filter. In a soil near neutrality both gelatin and the soil particles would be negatively charged. In consequence, maximum adsorption of the gelatin by the soil because of neutralizing opposite charges would not occur. Gieseking (*6*) lists "gelatine" among the exchangeable cations that react with montmorillonite and related minerals. As he used clays saturated with hydrogen ions, his findings might conceivably not apply to the more alkaline conditions near neutrality prevailing in these tests. Other factors undoubtedly contribute to the results observed.

The nitrogen of theobromine (dimethyl xanthine, a purine base) was completely retained in the top pot. Hodgman (*8*) lists caffeine (trimethyl xanthine) as being little stronger as a base than water. Assuming that there is small difference between these two compounds as bases, the complete retention of the nitrogen of theobromine in the top pot is somewhat surprising if considered from the viewpoint of its basic properties alone. Some unknown substance or substances in the soil solution might have precipitated the theobromine, or the soil solids might have reacted with it in such a way as to remove it from solution.

DISCUSSION

The nonretention of nitrogen in anionic units is not unexpected; and from the standpoint of the theory of cationic exchange in soils, the practically complete retention of the nitrogen in medium to strongly

basic cations is almost predictable. Considering, however, the retention of the nitrogen of the approximately neutral or amphoteric units, the behavior of any compound cannot be predicted on the basis of reaction alone. Thus though formamide is probably a weaker base than acetamide, its nitrogen was retained much more strongly by the soil solids than that of acetamide.

In the presence of proper catalysts or under conditions favoring microbial activity, ammonia may be more or less easily split off from these parent compounds. Sidgwick (*13, p. 141*) indicates that formamide is more easily hydrolyzed to ammonia and the corresponding organic acid than is acetamide. If ammonia were thus separated from any of these compounds during percolation, the soil would react with it in accordance with its own individual chemical properties and would retain it at, or very near, the point in the soil where that particular molecule of ammonia was split off. If such were the mechanism involved, a slower rate of percolation would allow a greater lapse of time for catalytic reaction or biological activity to become effective in splitting off more ammonia.

A reexamination of yield data disclosed approximately equal sums for the yields of three pots for given columns percolated with solutions of the same compound. Considerable variation occurred, however, among yields secured from the replications of the first, second, or third pots respectively from these columns. Chance variations in the rate of dripping from the reservoir above, together with unequal rates of percolation from pot to pot caused by chance differences in packing of the soil, might well have produced such variation. The writer considers this unequal rate of percolation as one major cause of high variability in some of these tests. In growth series 7, glycine, cysteine hydrochloride, and arginine hydrochloride were percolated by adding in five successive portions approximately one-fifth of the percolating solutions to the respective reservoirs, with about 12 hours between successive applications. Prolonging the time of percolation, as in growth series 7, apparently favored ammonium formation by catalytic reaction or microbial activity. See glycine in growth series 3 and 7, table 1, sec. C.)

Transformations of cysteine, which is very soluble, to cystine, which is very slightly soluble, may take place by oxidation in air or under alkaline conditions (*7, p. 123*). With the concentration used (approximately 20 millimols per liter), about 90 ercent of the nitrogen present would be precipitated if all the cysteine were changed to cystine. This transformation might well be one of the mechanisms by which such a large part of the nitrogen of cysteine was retained in the top pot. No mechanism of the same type seems to be at hand to explain the somewhat similar retention with glycine.

In case injury followed a particular test, it was assumed that the compound in question was toxic, although the damage may have been caused by decomposition products of that compound. Since the soils used were deficient in nitrogen under the conditions of the experiment, significant beneficial responses showed the position of the nitrogen of the compound regardless of whether this element was present in its original form or in physiologically effective forms resulting from subsequent biological changes.

It is assumed that many of these compounds if left to incubate a sufficiently long time would be converted almost quantitatively to nitrates. Such nitrates would, of course, be expected to react as have the nitrates reported in this paper.

Retention of the soil solids of some of the compounds tested, such as potassium ferrocyanide, potassium ferricyanide, and phenylurea as shown in table 1 was not fully determined by the criterion of subsequent plant response. Obviously these compounds were neither toxic enough nor beneficial enough in these experiments to influence the crop. Studies by the methods of analytical chemistry would undoubtedly be required for further advancement of our knowledge in this direction.

This study has served to give in terms of plant response a qualitative (and in some cases a semiquantitative) estimate of the reaction of the nitrogen of a wide variety of compounds in solution with the solid phase of the soil. Further elucidation of the role of various factors involved, especially with some of the compounds presenting new types of behavior, must await the accumulation of further experimental evidence.

SUMMARY

Each nitrogen compound in solution was allowed to percolate through a column of three pots containing dry soil deficient in nitrogen. The volume of solution was sufficient to wet all the soil but without great excess. If the compound was beneficial, the enhanced growth of the subsequent test crop was used to determine retention; if it was toxic, the reduced growth was used. The results obtained were as follows:

(1) Nitrogen contained in anionic units in the compounds: Calcium nitrate, sodium nitrate, sodium nitrite, potassium cyanide, and potassium cyanate were not retained by the solids in the soils tested, as was shown by beneficial plant response in all pots of the respective columns. Nonretention of sulfamic acid, picric acid, and sodium thiocyanate was shown by injury to the growth throughout the pots of the respective columns.

(2) Nitrogen contained in cationic units in the compounds: Ammonium acetate, ammonium carbonate, ammonium hydroxide, ammonium phosphate (monohydrogen), ammonium sulfamate, ammonium sulfate, hydrazine sulfate, hydroxylamine sulfate, tetramethylammonium chloride, and the hydrochlorides of trimethylamine, dimethylamine, methylamine, choline, and arginine were completely or almost completely retained in the top pots of their respective columns. Most of the nitrogen of semicarbazide hydrochloride and of guanidine carbonate was retained in the top pots of their respective columns; yet small but still significant amounts penetrated to lower pots.

(3) There was no evidence of any retention of the nitrogen of acetonitrile, acetoxime, or acetamide. The nitrogen of glycine, cysteine hydrochloride, glutamic acid, formamide, urea, and $CaCN_2$ was retained to a certain degree by the soil solids. Indications are that decomposition to liberate ammonia and the resultant retention of this form of nitrogen during the period of actual percolation are important factors in retention with these compounds. Peptone, egg

albumin, and blood albumin showed the same type of retention, whereas the nitrogen of gelatin and that of theobromine were completely retained in the top pots of their respective columns.

LITERATURE CITED

(1) CONANT, JAMES BRYANT.
1933. THE CHEMISTRY OF ORGANIC COMPOUNDS; A YEAR'S COURSE IN ORGANIC CHEMISTRY. 623 pp., illus. New York.

(2) CONRAD, JOHN P.
1939. RETENTION OF SOME PHOSPHORUS COMPOUNDS BY SOILS AS SHOWN BY SUBSEQUENT PLANT GROWTH. Jour. Agr. Res. 59: 507–518, illus.

(3) ——— and ADAMS, C. N.
1939. DETERMINING BY PLANT RESPONSE THE RETENTION OF NUTRIENT IONS BY SOILS. Amer. Soc. Agron. Jour. 31: 29–34, illus.

(4) CROSBY, STANLEY W., and CARPENTER, E. J.
1931. SOIL SURVEY OF THE DIXON AREA, CALIFORNIA. U. S. Dept. Agr., Bur. Chem. and Soils Ser. 1931, Rept. 7, 46 pp., illus.

(5) FISHER, R. A.
1932. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, rev. and enl., 307 pp., illus. Edinburgh and London.

(6) GIESEKING, J. E.
1939. THE MECHANISM OF CATION EXCHANGE IN THE MONTMORILLONITE-BEIDELLITE-NONTRONITE TYPE OF CLAY MINERALS. Soil Sci. 47: 1–13, illus.

(7) HAWK, PHILIP B., and BERGEIM, OLAF, in collaboration with OSER, BERNARD L., and COLE, ARTHUR G.
[1937]. PRACTICAL PHYSIOLOGICAL CHEMISTRY. Ed. 11, 968 pp., illus. Philadelphia.

(8) HODGMAN, CHARLES D., ed. in chief.
[1935]. HANDBOOK OF CHEMISTRY AND PHYSICS; A READY-REFERENCE BOOK OF CHEMICAL AND PHYSICAL DATA. Ed. 20, 1951 pp., illus. Cleveland.

(9) HOLMES, L. C., ECKMANN, E. C., NELSON, J. W., and GUERNSEY, J. E.
1920. RECONNAISSANCE SOIL SURVEY OF THE MIDDLE SAN JOAQUIN VALLEY, CALIFORNIA. U. S. Dept. Agr., Bur. Soils Field Oper. 1916, Rpt. 18: 2421–2529, illus.

(10) NATIONAL RESEARCH COUNCIL.
1926–33. INTERNATIONAL CRITICAL TABLES OF NUMERICAL DATA, PHYSICS, CHEMISTRY, AND TECHNOLOGY . . . 7 v. and index. New York.

(11) OLANDER, ARNE.
1927. THE KINETICS OF OXIME FORMATION. Ztschr. f. Phys. Chem. 129 1–32. [Original not seen. Abstract in Chem. Abs. 22: 344. 1928.]

(12) RUSSELL, SIR E. JOHN.
[1937.] SOIL CONDITIONS AND PLANT GROWTH. Ed. 7, 655 pp., illus. London, New York [etc.].

(13) SIDGWICK, N. V.
1937. THE ORGANIC CHEMISTRY OF NITROGEN. New ed., rev. and rewritten by T. W. J. Taylor, and Wilson Baker. 590 pp., illus. Oxford, England.

THE RESISTANCE OF PROGENY OF KATAHDIN POTATOES TO VIROSES [1]

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INTRODUCTION

Schultz et al. (*11*) [3] reported the Katahdin variety of potato (*Solanum tuberosum L.*) as being resistant to infection in the field by the mild mosaic virus and by the latent or X virus. They further reported the Katahdin variety to be resistant to the latent virus when mechanical inoculation methods were used, but susceptible when tested by tuber grafts. Smith (*14*), however, reported that Katahdin is not resistant to the X and Y viruses when inoculated by the rubbing and needle-scratch methods. In a previous publication (*9*) it was shown that Katahdin, as well as Chippewa and Russet Burbank, was somewhat resistant to infection by the Y virus in the field when compared with Bliss Triumph, Gold Coin, Irish Cobbler, Warba, and U. S. D. A. 41956. It was further shown that Katahdin was the only variety that transmitted to seedling progeny resistance to infection by the Y virus in field tests of seedlings developed from crossing and selfing 11 varieties of potatoes.

The Y virus in combination with the X virus is responsible for the rugose mosaic disease that is very destructive, in many potato-growing areas, to common commercial varieties of potatoes. Since the older commercial varieties in the United States are carriers of the X virus without material damage to the stock, the introduction of the Y virus is the destructive element in rugose mosaic (*6*).

It has been shown in a previous publication (*9*), as well as by other investigators, that the Y virus is capable of causing severe damage to potatoes even in the absence of the X virus. This has been demonstrated by inoculation of the Y virus into seedlings that were virus-free. Marked variation in symptoms, including mottling and severe necrosis, is exhibited by seedlings and clones infected only by the Y virus (*9*).

In order to obtain further information on the value of Katahdin as a parent in the development of potato seedlings resistant to viroses, a number of tests were made beyond those reported in 1937 (*9*). The results of these tests are reported in the present paper.

RESISTANCE TO VEIN-BANDING

METHODS

Seed developed by the Division of Horticulture of the State College of Washington, as well as seed and tubers of named and numbered varieties furnished by the United States Department of Agriculture, were used in these investigations.

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[2] Acknowledgement is made to Dr. F. J. Stevenson of Bureau of Plant Industry, U. S. Department of Agriculture, for furnishing seed and tubers of named and numbered varieties for the tests.
[3] Italic numbers in parentheses refer to Literature Cited, p. 644.

The seed was planted in the greenhouse about March 15 each season and the young seedlings transplanted to beds 4 to 6 weeks after seeding. Previous to 1938 all the seedlings were transplanted to the field during the last of May or the first part of June. In 1938 and 1939 the seedlings were mechanically inoculated with the Y virus according to the method described by Jones (*8*) soon after they were transplanted to the beds in the greenhouse. A period of 30 to 45 days was allowed following inoculation before roguing the affected plants and transplanting the symptomless (resistant) plants to the field.

The year the seedlings were started and the numbers observed are as follows: 1924, 167; 1925, 28; 1926, 138; 1927, 924; 1928, 443; 1929, 448; 1930, 449; 1931, 350; 1932, 720; 1934, 2,081; 1936, 776; 1937, 4,000; 1938, 9,728; and 1939, 8,038. Records of the prevalence of vein-banding (Y virus) in the plantings have been taken each year since 1933 by making three field inspections during the growing season. Previous to 1933 the badly diseased stock was rogued from the planting without definite information on the causal virus. During the winters of 1933 to 1937 all the virus-free clones, those recorded as "virus infection questionable," and many of the virus-infected clones, as recorded in the previous summer observations, were indexed in the greenhouse. Five to twenty tubers representing each clone to be tested were planted in the greenhouse. The indexing consisted in observing the symptoms of disease, if any, shown by each plant, and inoculating with macerated leaf tissue from each potato plant into tobacco plants according to the method described by Jones (*8*). These detailed studies in the greenhouse showed that the Y virus was responsible for practically all of the viroses observed in the field plantings with the exception of a few cases of leaf roll and latent mosaic. In 1938 and 1939 curly top developed in the plantings to a damaging extent.

In addition to the seedlings started each year, field plantings were made with the clones noted the previous year as resistant to the Y virus. Clones that had been grown in the field 2 or more years were planted with 25 hills per numbered variety, often in triplicate, and the clones that had been grown only 1 year from true seed were planted with 12 hills per numbered variety. Many of the numbered varieties noted as susceptible to vein-banding, but having desirable characters, were saved each season and interplanted in the rows with the resistant varieties. Twenty-five hills each of the named and numbered varieties, often in triplicate, and the single drop tubers (1 small tuber from each numbered variety) furnished by the United States Department of Agriculture were interplanted in rows with the stock developed from seed.

Most seasons every third or fifth row was planted with varieties known to be affected with vein-banding, and from 40 to 50 percent of the stock used each season was affected with vein-banding. Although the resistant clones and varieties were not uniformly interplanted with the diseased stock in the rows, observations each season showed that ample opportunity was afforded for 100-percent infection of the susceptible clones and numbered varieties.

Varieties which showed no vein-banding or less than 30 percent affected in a given season were classed as resistant, in contrast to the

susceptible ones, most of which showed 100-percent infection under similar conditions.

EFFECT OF A COMBINATION OF VIRUSES

In order to determine whether a combination of viruses might be responsible for the variation in symptoms, a series of tests was made on different lots of seedlings using the virus extract from necrotic (fig. 1) as well as from mottled (fig. 2, *C*) plants.

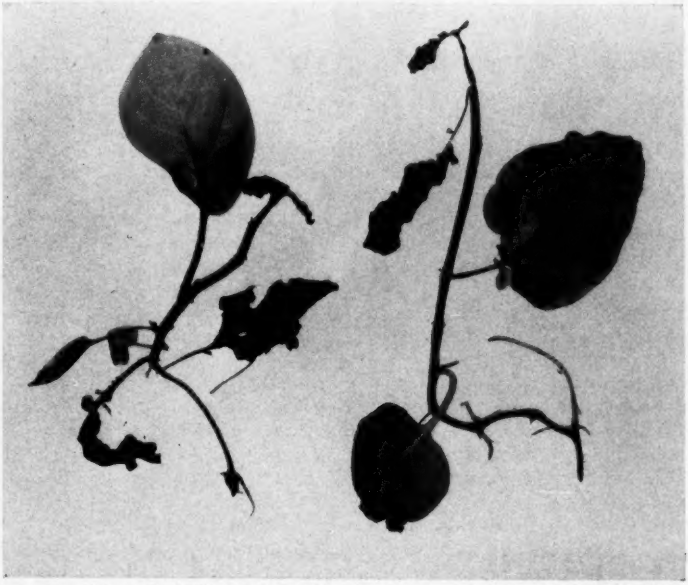

FIGURE 1.—Necrosis of Katahdin, selfed seedlings produced by inoculation with the Y virus.

TABLE 1.—*Variation of symptoms in potato seedlings following mechanical inoculation with the Y virus*

Source of virus	Parentage of seedlings inoculated	Seedlings inoculated	Plants infected that showed—	
			Mottling	Necrosis
		Number	*Percent*	*Percent*
Mottled seedling in field [1]	Early Norther × McCormick	103	91.5	6.2
	Katahdin, selfed	103	18.4	13.6
Necrotic Katahdin seedling [2]	Early Norther × McCormick	36	91.7	8.3
	Keeper, selfed	16	100.0	0
	Irish Cobbler × Keeper	40	100.0	0
	Katahdin, selfed	45	35.6	33.4
Mottled Katahdin seedling [3]	Early Norther × McCormick	38	92.1	7.9
	Keeper, selfed	15	100.0	0
	Irish Cobbler × Keeper	43	100.0	0
	Katahdin, selfed	45	35.6	28.8

[1] The Y virus was obtained from infected tobacco plants that had been inoculated from seedling 2563 tissue collected in the field.
[2] The Y virus was transferred from necrotic Katahdin seedlings noted above.
[3] The Y virus was transferred from mottled Katahdin seedlings of second test noted above.

An analysis of the results of these tests (table 1) shows that the ratio of necrosis to mottling was practically the same in infected plants of seedling lots regardless of whether the inoculum was from necrotic or mottled plants. This shows that a single Y virus was responsible for the variable symptoms observed.

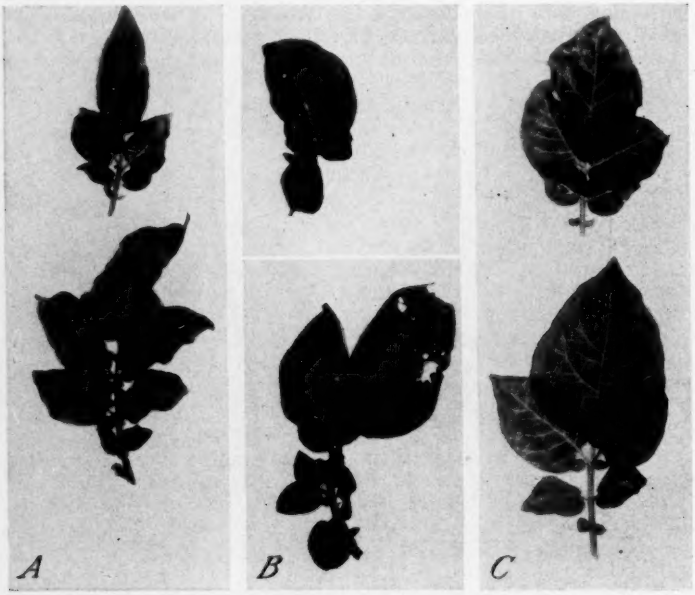

FIGURE 2.—Symptoms produced on potato seedlings: *A*, Mottling; *B*, necrosis by the tobacco mosaic virus; *C*, mottling by the Y virus.

TABLE 2.—*The effect of vein-banding upon yields of potato varieties, Pullman, Wash., 1938*

Variety	First year in field [1]			Second year in field [2]		
	Hills observed	Average yield per hill		Hills observed	Average yield per hill	
		Total	U. S. No. 1		Total	U. S. No. 1
	Number	*Pounds*	*Pounds*	*Number*	*Pounds*	*Pounds*
U. S. D. A. 46000	71	2.0	1.7	69	0.5	0.4
Earlaine	64	2.4	2.0	53	1.0	.7
Houma	64	2.5	1.9	70	.7	.3
Katahdin	69	3.8	2.9	55	3.6	2.7

[1] New stock obtained from the U. S. Department of Agriculture for 1938 planting. Field records early in the growing season showed no vien-banding.

[2] Stock saved from 1937 trial plots in Pullman. The Katahdin variety was carefully rogued in 1937, 35 percent of the plants having become affected with vein-banding. The other varieties became 100 percent affected with vein-banding in 1937 and were not rogued.

EFFECT OF VEIN-BANDING ON TUBER YIELDS

The effect of vein-banding on tuber yields was obtained during the season of 1938 on a number of varieties in the field planting, as shown in table 2. An analysis of the results of these tests shows that vein-banding reduced the yields of U. S. D. A. 46000, Earlaine, and Houma 75, 58, and 72 percent, respectively, in the second season following 100-percent infection with the virus. Only 5-percent reduction in yield was noted in the rogued Katahdin stock.

RESISTANCE OF SEEDLINGS TO MECHANICAL INOCULATION

In order to reduce the population to be planted in the field and to determine if mechanical inoculation could be used as a criterion of susceptibility, the seedlings that were started in 1938 and 1939 were mechanically inoculated with the Y virus in the greenhouse soon after they were transplanted to the beds. A period of 30 to 45 days was allowed before roguing the affected plants and transplanting the symptomless (resistant) plants to the field. Since the susceptible plants showed symptoms in 15 to 25 days following inoculation, all plants that would develop symptoms from the mechanical inoculation probably showed the infection and were rogued in the greenhouse. Observations made 10 to 12 days after the symptomless plants were placed in the field failed to show any affected plants which indicates that only healthy plants or possibly those with masked symptoms were taken into the field.

The results recorded in table 3, where the tests are summarized according to the parents used in seed production, show that some lots of seedlings were 100 percent susceptible to mechanical inoculations with the Y virus, while others were somewhat resistant; but none of the seedling lots showed immunity to infection. Seedling lots produced from seed of which Katahdin was a parent consistently showed some resistance to infection. Based on Katahdin selfed seedlings showing 54-percent resistance, it appears that higher percentages of resistance were obtained by crossing Katahdin with Ackersegen, Imperia, Sebago, and U. S. D. A. 41956, and that reduced resistance was obtained by crossing Katahdin with Earlaine-Sebec, Earlaine-Charles Downing, and Houma. The use of Green Mountain, No Blight, and Albion in crosses with Katahdin did not materially change the resistance of the seedlings as compared with Katahdin, selfed seedlings.

RESISTANCE OF SEEDLINGS TO FIELD INOCULATION

The results of the field tests with seedlings (table 3) show that mechanical inoculation with the Y virus does not record the complete susceptibility of seedling lots since greater susceptibility was observed in the field beyond that noted in the greenhouse following mechanical inoculation. Mechanical inoculation in the greenhouse, however, appears to indicate the relative susceptibility of seedling lots.

Only seedlings having Katahdin as a parent showed resistance to vein-banding in the field. Crossing Katahdin with Sebago or U. S. D. A. 41956 appeared to increase the percentage of resistance over that of Katahdin, selfed seedlings, but a consideration of the standard deviation shows that these increases are not significant.

TABLE 3.—*Summary of tests to determine the resistance of potato seedlings to vein-banding* [1]

Parentage of seedlings	Seed lots tested	Seedlings and clones observed [2]	Seedlings inoculated in the greenhouse [3]		Clones resistant in the field [4]	Clones rogued for curly top [5]
			Observed	Resistant		
	Number	*Number*	*Number*	*Percent*	*Percent*	*Percent*
McCormick:						
Selfed	1	65			0.0	
Early Norther	11	1,100	177	4.0	.0	
Keeper	4	131			.0	
Keeper, Early Norther	2	117			.0	
Keeper:						
Selfed	3	466	131	.0	.0	
Jersey Red Skin	1	85			.0	
American Wonder	3	100			.0	
Cobbler	3	252	183	.0	.0	
Burbank, selfed	9	1,342			.0	
Cobbler, selfed	4	470			.0	
Earlaine:						
Selfed	3	1,077	1,077	.0		
U. S. D. A. 41956	1	870	870	54.4	.0	20.1
No Blight	1	(360)	360	43.4	(.0)	17.3
Late Cobbler	1	61			.0	
Hindenburg, Ostragis	1	(198)	198	55.4	(.0)	6.9
Katahdin:						
Selfed	28	4,754 (180)	2,739	[6] 54.1±6.8	[6] 4.4±2.68	[6] 32.4±21.0
Green Mountain	4	3,765(1,033)	3,765	44.5	2.8(0)	13.8
No Blight	7	1,568 (576)	813	58.5	4.9 (2.3)	20.4
Sebago	2	612 (72)	612	74.8	8.2 (1.4)	59.
U. S. D. A. 41956	4	1,145 (354)	1,145	72.6	7.4 (4.2)	35.0
Houma	2	1,740 (540)	1,740	27.4	1.2 (.7)	10.1
Ackersegen	1	(58)	58	74.0	(.0)	.0
Imperia	1	(493)	493	82.8	(3.4)	34.8
Albion	1	(473)	473	55.2	(.8)	16.8
Earlaine, Sebec	2	455 (303)	455	29.0	.0(.0)	20.0
Earlaine, Charles Downing	1	(432)	432	5.3	(.0)	38.9
Carmen No. 3, Bussola, Rural New Yorker No. 2	7	2,766(1,598)	2,695	13.8	2.3 (.0)	36.1
Yellow-fleshed variety from Costa Rica, Aroostook Wonder, Sutton Flourball, Rural New Yorker No. 2, Bussola	6	84	84	48.8	4.7	18.2

[1] The following crosses with than 50 seedlings in each lot were also observed in the field and failed to show any clones resistant to vein-banding. McCormick crossed with American Wonder, Beauty of Hebron, Burbank, Early Norther, Early Six Weeks, Irish Cobbler, Jersey Red Skin, Keeper, Late Rose, McKinley, and Mortgage Lifter. Keeper crossed with Burbank. Late Rose, selfed, and crossed with American Wonder and Burbank. Early Norther, selfed. Katahdin crossed with American Wonder, Burbank, Chippewa, Early Norther, Eureka, Irish Cobbler, Keeper, and McCormick.

[2] Figures in parentheses show data collected on tests made for only 1 season. All other figures indicate that data are summaries of observations through 2 or 3 seasons.

[3] The seedlings were mechanically inoculated with the vein-banding virus in the greenhouse. A period of 30 to 45 days was allowed following inoculation before roguing the diseased plants and transplanting the symptomless (resistant) plants to the field.

[4] Clones showing less than 30 percent of infection were classed as resistant, and the percentage of clones resistant in the field was calculted on the total number of seedlings and clones of each parentage that was observed in the greenhouse and the field.

[5] Curly top caused by the leafhopper-transmitted virus that is associated with curly top of beets became a factor in the experimental plantings in 1938 and 1939. The percentage of clones rogued for curly top was calculated on the number of clones exposed to infection in the field. Lack of data shows that the tests were made previous to 1938 before curly top appeared as an important factor in the experimental plantings.

[6] Standard deviation of the mean. $S.D. = \sqrt{\frac{\Sigma d^2}{n-1}}$.

As was shown in the mechanical-inoculation tests, Earlaine-Sebec and Earlaine-Charles Downing crossed with Katahdin reduced the resistance of the seedlings in comparison with that obtained in seedlings from Katahdin, selfed parentage.

An example of the resistance shown in the field tests may be stated as follows: Of the 776 seedlings started in 1936, 89 were saved as resistant or promising and grown again in the field in 1937. The observations in the field in 1937, as well as indexing 5 tubers per clone

in the greenhouse during the winter of 1937–38, showed that only 18 of the 89 clones continued to show high resistance to vein-banding. The 18 resistant clones were grown in the field in 1938 and again in 1939 and were carefully rogued each season. Field observations on the 18 varieties during the last 2 seasons have shown vein-banding as follows: In 1938, 7 were virus-free, 6 showed less than 10 percent, and 5 showed less than 30 percent of the plants infected; in 1939, 7 were virus-free, 5 showed less than 10 percent, and 6 showed less than 40 percent of infection.

RESISTANCE OF POTATO VARIETIES TO FIELD INOCULATION

A quantity of tubers, as well as single drops of named and numbered varieties of potatoes recently developed by the United States Department of Agriculture, were planted in the same planting with the seedlings. If a quantity of the tubers of the varieties was furnished, 25 hills per variety were planted in triplicate. The following varieties became 100 percent affected with vein-banding in 2 years: Earlaine, Golden, Houma, 41956, 46000, 46110, 46125, 44537, 47154, and 47160. Under similar conditions the percentage of infection for the following varieties was: 46842, 0; 47208, 0; Chippewa, 1.9; 47196, 2.8; Katahdin, 12.9; 47296, 13.2; 336–123, 19.4; and Sebago, 31.5 .

The results of tests with single drop tubers of numbered varieties recorded in table 4 show that selfing Earlaine or crossing Earlaine with Katahdin or U. S. D. A. 41956 gave seedlings of reduced resistance as compared with seedlings produced from selfing Katahdin or crossing Katahdin with No Blight or U. S. D. A. 41956. These results are in accord with those obtained by mechanical inoculation and by field tests with seedlings.

SEED TRANSMISSION OF THE Y VIRUS

Observations on the seedlings produced in the greenhouse (tables 1 and 3), many of which were grown from seed collected from vein-banding-affected plants, failed to show any indication that the Y virus is seed transmitted.

TABLE 4.—*Tests with single drops of numbered varieties to determine resistance to vein-banding, Pullman, Wash., 1939*

Parentage of single-drop tubers [1]	Hills observed	Hills virus-free [2]
	Number	*Percent*
Earlaine, selfed	29	0.0
Earlaine × U. S. D. A. 41956	32	3.1
Earlaine × Katahdin	80	5.0
Katahdin, selfed	37	18.1
Katahdin × No Blight	88	21.6
Katahdin × U. S. D. A. 41956	74	25.7

[1] Single-drop tubers of numbered varieties furnished by the U. S. Department of Agriculture.
[2] Planting observed and rogued 3 times during growing season. The last observation was made Oct. 6, 1939, 3 days before harvest.

RESISTANCE TO CURLY TOP

A serious disease appeared in the potato planting in 1938 and 1939 which showed as dwarfing and erectness of the plants, rolling, harsh-

ness, and reddening of the foliage, and extreme dwarfing and increase in the number of tubers (fig. 3). The disease was very severe in the seedling planting, often killing the affected plants and making it

FIGURE 3.—Curly top of Katahdin, selfed seedling from experimental plot, 1939.

difficult to obtain definite data on susceptibility to vein-banding. The general appearance of the disease resembled that associated with psyllid yellows or aster yellows, but frequent observations and the collection of prevalent insects failed to show the presence of the insect

vectors of these two diseases. The fact that aster yellows has never been recorded in the immediate vicinity of Pullman is further evidence that the aster yellows virus was not the causal agent.

The prevalence of curly top in tomato plantings adjacent to the potatoes and the heavy infestation of *Eutettix tenellus* (Baker) on the potato plants indicated that the curly top virus was responsible for the symptoms. The virosis known as curly top of beet, which is transmitted by the leafhopper *E. tenellus*, has been reported on potato by Severin (*13*) who states: "Further experiments will show whether tuber transmission of curly top occurs in potato."

Tubers collected in the field from 25 potato plants affected with curly top were planted in the greenhouse in the winter of 1938–39. This stock produced 3 plants showing spindly sprouts, erectness, inward rolling, and yellowing of foliage which indicates only a low percentage of tuber transmission. Healthy tomato scions were grafted onto the affected potato plants, and typical symptoms of the common tomato curly-top disease developed on the tomato.

The data collected on the prevalence of curly top in the seedlings (table 3) show wide variation; however, the large standard deviation of the mean indicates no significant differences in susceptibility of the seedling lots.

Curly top of tomatoes has been prevalent adjacent to the potato plots each year since the potato project was started. Observations in commercial and garden plantings of the Katahdin variety in the vicinity of Pullman, in 1938 and 1939, showed 8 to 10 percent of the plants affected with curly top. The fact that the disease was not important in the potato planting until 1938 and 1939 coincident with the general use of Katahdin as a parent in the seedlings would indicate that possibly Katahdin and Katahdin progeny are more susceptible than other varieties.

RESISTANCE TO TOBACCO MOSIAC

Allard (*1*), Dickson (*4*), Clinton (*3*) and Schultz and Folsom (*12*) have reported unsuccessful attempts to infect potatoes with the tobacco mosaic virus. Johnson (*7*) obtained local necrotic lesions on the inoculated leaves of Bliss Triumph but concluded that tobacco mosaic did not become systemic in the potato. Fernow (*5*) reported symptoms on potato closely parallel to those of streak of potato and obtained systemic infection with tobacco mosaic on the Green Mountain variety. Blodgett (*2*) showed that tobacco mosaic produced different symptoms on certain varieties of potatoes, the Rural New Yorker No. 9 being especially susceptible, and that on some varieties already affected with the latent virus, streak symptoms appeared. Folsom and Bonde (*6*) have shown that tobacco mosaic can infect Green Mountain potato plants, producing symptoms rather like those of the potato streak disease.

The susceptibility of a number of potato varieties and seedlings to infection by the tobacco mosaic virus has been determined by inoculation in the greenhouse of the State College of Washington. Tobacco mosaic virus No. 1 obtained from Dr. James Johnson of the University of Wisconsin was used in all of the tests. The virus was inoculated into tobacco plants in 1929, and the diseased plants were harvested and hung in a shed adjacent to the greenhouse. Portions of this

material which had been dried for 10 years were used to inoculate young tobacco plants, and the newly affected tobacco plant tissue was used as inoculum on the potato plants, according to the method described by Jones (*8*) supplemented by the sprinkling of carborundum dust on the foliage to be inoculated previous to inoculation (*10*).

The results of inoculations (table 5) show that plants of the Rural New Yorker No. 9 variety and seedlings produced from crosses of Early Norther × McCormick and Irish Cobbler × Keeper were somewhat susceptible, but that plants of the varieties Early Rose, Chippewa, Green Mountain, Irish Cobbler, Katahdin, and seedlings from selfed Earlaine, selfed Katahdin, selfed Keeper, and Katahdin crossed with Houma, Sebago, or U. S. D. A. 41956 were immune to tobacco mosaic when mechanical-inoculation methods were used. The symptoms shown by potato seedlings affected with tobacco mosaic varied from distinct mottling with large, raised, dark-green areas (fig. 2, *A*) to extreme necrosis (fig. 2, *B*) depending upon the reaction of the individual susceptible seedling. Affected plants of the Rural New Yorker No. 9 variety showed leaf and stem necrosis similar to potato streak symptoms.

TABLE 5.—*Summary of tests to determine resistance of potato varieties and seedlings to tobacco mosaic* [1]

Variety or parentage of seedling	Plants inoculated	Plants infected	Variety or parentage of seedling	Plants inoculated	Plants infected
	Number	*Percent*		*Number*	*Percent*
Early Rose	31	0.0	Early Norther × McCormick	538	19.3
Chippewa	2	.0	Irish Cobbler × Keeper	179	4.5
Green Mountain	33	.0	Katahdin, selfed	444	.0
Irish Cobbler	33	.0	Katahdin × Houma	108	.0
Katahdin	408	.0	Katahdin × Sebago	108	.0
Rural New Yorker No. 9	20	15.0	Katahdin × U. S. D. A. 41956	108	.0
Earlaine, selfed	108	0.0	Keeper, selfed	48	.0

[1] Tobacco mosaic virus No. 1a from tobacco plant that had been inoculated from tobacco tissue that had been dried for 10 years. An equal number of uninoculated control plants in each test failed to show symtoms of tobacco mosaic.

During the winter of 1937–38, 10 tomato scions were grafted onto 10 selfed Katahdin seedlings. The tomato scions were subsequently inoculated with tobacco mosaic. The symptoms of tobacco mosaic appeared on the tomato foliage, and definite mottling with large dark-green areas followed by necrosis of the light-green areas showed on the foliage of the potato seedlings. Inoculations from the affected potato foliage to tobacco plants showed the presence of tobacco mosaic in the potato foliage. Tubers collected from the mosaic-affected selfed Katahdin potato plants were planted in the greenhouse along with tubers collected from the Early Norther × McCormick seedlings affected by tobacco mosaic. The plants produced from the Early Norther × McCormick seedling tubers showed strong symptoms of tobacco mosaic but those from selfed Katahdin tubers failed to show any symptoms of the disease. Inoculations with potato plant tissue from the 2 lots of plants verified the presence of the tobacco mosaic virus in the Early Norther × McCormick seedlings and its absence from the selfed Katahdin seedlings. These results show that the selfed Katahdin seedlings were susceptible to infection by the tobacco mosaic virus through grafts with mosaic-affected tomato scions but

that the virus was not transmitted through the tubers produced by affected plants.

RESISTANCE TO LATENT MOSAIC

Schultz et al. (*11*) report that leaf-rubbing, tuber-grafting, and field-contact tests failed to produce infection by the latent or X virus in the potato variety U. S. D. A. 41956. The same authors further report that Katahdin was resistant to latent mosaic in field tests, but contracted the disease in tuber-graft tests where it was expressed as top necrosis. The report of Smith (*14*) shows Katahdin to be susceptible to infection by the X virus by the rubbing and leaf-scratch methods of inoculation, the resultant disease showing as circular necrotic lesions followed by a mild mottling of the topmost leaves, while the other leaves developed a curious network of faint necrotic lines most pronounced on the under surfaces.

Inoculations to determine the susceptibility of Katahdin to infection by the X virus have been made at different times over a period of years. In the earlier tests with the X virus from Russet Burbank and Green Mountain plants, Katahdin was shown to be immune to infection by mechanical inoculation (table 6).

TABLE 6.—*Summary of tests to determine the resistance of Katahdin to mechanical inoculation with the latent or X virus*

Original source of the virus	Source of virus for the inoculation test	Katahdin plants in test	Plants infected by the latent virus	
			Number[1]	Symptoms
		Number		
Russet Burbank	Russet Burbank	19	0	None.
Do	Tobacco	10	0	Do.
Green Mountain	do	10	0	Do.
Seedling 2277	do	4	4	Mottling, crinkling.
Do	Katahdin	15	15	Do.
Do	*Datura stramonium*	20	20	Do.
Control	Uninoculated	24	0	None.

[1] Based on symptoms shown by each plant and inoculations to tobacco plants with potato plant tissue, alone and in combination with the vein-banding virus.

During the winter of 1933–34, four Katahdin plants were inoculated with a virus that appeared in seedling 2277, and very definite mottling of the foliage resulted (fig. 4). Further tests showed that the causal virus was definitely of the X type based on the following characters: Thermal inactivation, 68 to 70° C.; produced spot necrosis of tomato and tobacco in combination with the vein-banding virus; caused systemic infection in *Datura stramonium* (fig. 5); and retained all of its original characters after passage through *D. stramonium*.

In further tests on Katahdin this variant of the X virus consistently produced 100-percent infection in mechanical inoculations. The disease has been perpetuated through three generations of tubers and continues to show the same severe mottling without necrosis. The disease has been observed only on the experimental plants in the greenhouse. The original source of the virus is unknown beyond the fact that it appeared in plants of seedling 2277 developed from Early Norther × McCormick.

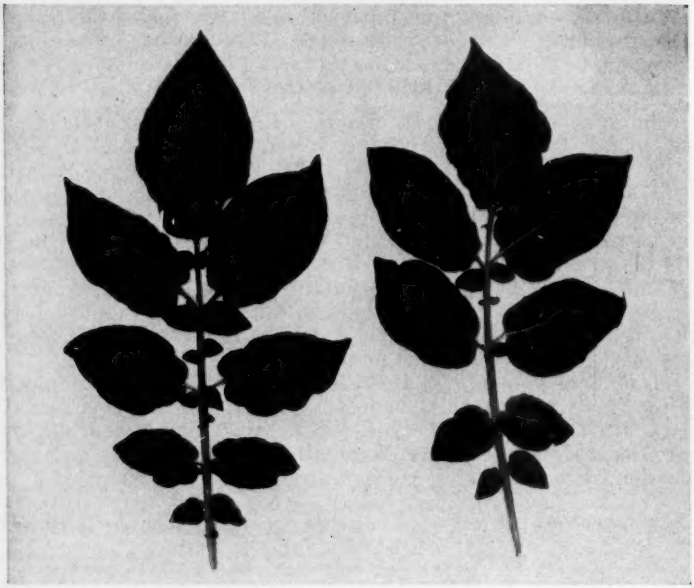

FIGURE 4.—Mottle of Katahdin leaves produced by inoculation with the X-type virus obtained from seedling 2277.

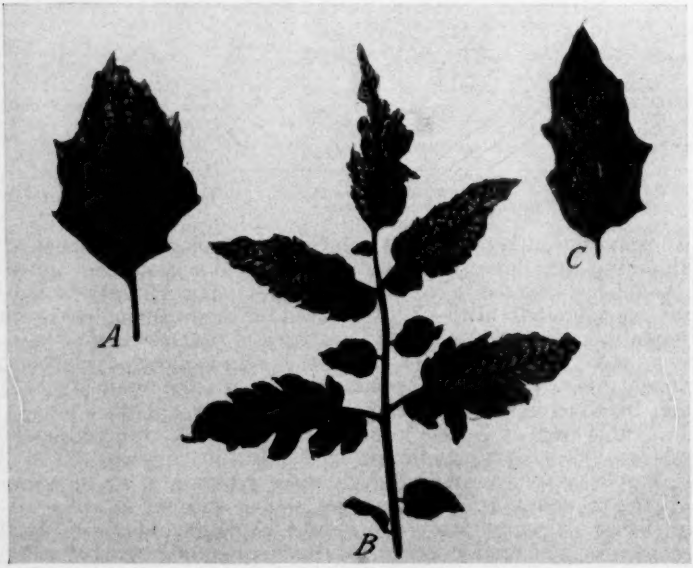

FIGURE 5.—Mottle of *Datura stramonium* (*A*) and tomato leaves (*B*) produced by inoculation with the X-type virus obtained from potato seedling 2277 compared with mottle of *D. stramonium* leaf (*C*) produced by inoculation with the X virus from Green Mountain potato.

The tests with the X virus, as shown in table 6, show that Katahdin is resistant to infection by the common X virus found in Russet Burbank and Green Mountain potatoes, but susceptible to the X virus from seedling 2277.

SUMMARY

The vein-banding or Y virus, which is the destructive element in rugose mosaic, produces marked variation in symptoms depending on the reaction of the individual seedling and may reduce yields of susceptible varieties as much as 75 percent.

Some lots of seedlings were 100 percent susceptible to mechanical inoculation with the Y virus, while others were somewhat resistant and none of them showed immunity. Seedling lots, produced from seed of which Katahdin was a parent, consistently showed some resistance to infection by mechanical inoculation with the Y virus; and crosses of Katahdin with Ackersegen, Imperia, Sebago, and U. S. D. A. 41956 appeared to transmit greater resistance than did selfing Katahdin.

Mechanical inoculation with the Y virus did not show the complete susceptibility of seedling lots since greater susceptibility was observed from natural inoculation in the field.

Only seedlings having Katahdin as a parent showed resistance to vein-banding in the field, and no significant increase in resistance over that shown by selfed Katahdin was obtained by crossing Katahdin with other varieties.

Many named and numbered varieties of potatoes became 100 percent affected with vein-banding in two seasons in the field, while others, including Katahdin, showed less than 32-percent infection under similar conditions.

Tests with single-drop tubers showed that selfing Earlaine or crossing Earlaine with Katahdin or U. S. D. A. 41956 gave seedlings with reduced resistance to the Y virus as compared with seedlings produced by selfing Katahdin or crossing Katahdin with No Blight or U. S. D. A. 41956.

Observations on thousands of seedlings produced from seed collected from vein-banding-affected plants fail to show any seed transmission of the Y virus.

The virus which causes curly top of beets was very prevalent in the field, but no significant difference in susceptibility was noted in the seedling lots.

Plants of the Rural New Yorker No. 9 variety and seedlings produced from crosses of Early Norther × McCormick and Irish Cobbler × Keeper were susceptible to mechanical inoculation with tobacco mosaic, but all the other varieties tested, including Katahdin and other seedlings including crosses with Katahdin, were immune.

Selfed Katahdin seedlings were susceptible to infection by the tobacco mosaic virus through grafts with mosaic-affected tomato scions, but the virus was not transmitted through tubers collected from affected plants.

Katahdin was immune to infection by mechanical inoculation with the X virus from the Russet Burbank and Green Mountain varieties, but was susceptible to a variant of the X virus obtained from potato seedling 2277.

LITERATURE CITED

(1) ALLARD, H. A.
1914. THE MOSAIC DISEASE OF TOBACCO. U. S. Dept. Agr. Bul. 40, 33 pp., illus.
(2) BLODGETT, F. M.
1927. TOBACCO MOSAIC ON POTATOES. Phytopathology 17: 727–734.
(3) CLINTON, G. P.
1915. REPORT OF THE BOTANIST FOR 1914. CHLORISIS OF PLANTS WITH SPECIAL REFERENCE TO CALICO OF TOBACCO. Conn. State Agr. Expt. Sta. Rpt. 1914: [357]–424, illus.
(4) DICKSON, B. T.
1922. STUDIES CONCERNING MOSAIC DISEASES. Macdonald Col. Tech. Bul. 2, 125 pp., illus.
(5) FERNOW, K. H.
1925. INTERSPECIFIC TRANSMISSION OF MOSAIC DISEASES OF PLANTS. N. Y. (Cornell) Agr. Expt. Sta. Mem. 96, 34 pp., illus.
(6) FOLSOM, DONALD, and BONDE, REINER.
1937. SOME PROPERTIES OF POTATO RUGOSE MOSAIC AND ITS COMPONENTS. Jour. Agr. Res. 55: 765–783, illus.
(7) JOHNSON, JAMES.
1927. THE CLASSIFICATION OF PLANT VIRUSES. Wis. Agr. Expt. Sta. Res. Bul. 76, 16 pp., illus.
(8) JONES, L. K.
1932. A NEW METHOD OF INOCULATING WITH VIRUSES. (Abstract) Phytopathology 22: 998–999.
(9) ——— and VINCENT, C. L.
1937. THE SUSCEPTIBILITY OF POTATOES TO THE VEIN-BANDING VIRUS. Jour. Agr. Res. 55: 69–79, illus.
(10) RAWLINS, T. E., and TOMPKINS, C. M.
1936. STUDIES ON THE EFFECT OF CARBORUNDUM AS AN ABRASIVE IN PLANT VIRUS INOCULATIONS. Phytopathology 26: 578–587, illus.
(11) SCHULTZ, E. S., CLARK, C. F., BONDE, REINER, RALEIGH, W. P., and STEVENSON, F. J.
1934. RESISTANCE OF POTATO TO MOSAIC AND OTHER VIRUS DISEASES. Phytopathology 24: 116–132.
(12) ——— and FOLSOM, DONALD.
1923. TRANSMISSION, VARIATION, AND CONTROL OF CERTAIN DEGENERATION DISEASES OF IRISH POTATOES. Jour. Agr. Res. 25: 43–118, illus.
(13) SEVERIN, HENRY H. P.
1929. ADDITIONAL HOST PLANTS OF CURLY TOP. Hilgardia 3: [595]–636, illus.
(14) SMITH, KENNETH M.
1933. RECENT ADVANCES IN THE STUDY OF PLANT VIRUSES. With foreword by F. T. Brooks. 423 pp., illus. London.

U. S. GOVERNMENT PRINTING OFFICE: 1940